EFFECTS OF PETROLEUM HYDROCARBONS ON ALASKAN AQUATIC ORGANISMS: A COMPREHENSIVE REVIEW OF ALL OIL-EFFECTS RESEARCH ON ALASKAN FISH AND INVERTEBRATES CONDUCTED BY THEAUKE BAY LABORATORY, 1970-81

by

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SUMMARY

This report reviews and summarizes all oil-effects research by the Auke Bay Laboratory from the beginning of these studies in 1970 through 1981. Both published and unpublished results from 62 studies are included, regardless of funding source. Research is reviewed according to subject (e.g., toxicity, sublethal effects, studies at Port Valdez). A bibliography and abstracts are also included.

Studies With Crude Oil,
No. 2 Fuel Oil, and Their Components

Results from different studies should be compared with caution because the comparisons can be misleading if exposure methods, chemical analyses, test animals, or life stages are different. Temperature also influences results of studies by affecting evaporation and biodegradation of petroleum hydrocarbons. Some generalizations, however, can be made from the results of our studies. The water-soluble fraction (WSF) of No. 2 fuel oil is consistently more toxic than the WSF of crude oil even though the WSF'S of fuel oil contain low concentrations of monoaromatic hydrocarbons. The total toxicity of individual aromatic hydrocarbons in the WSF'S did not, however, account for all of the toxicity of the oils; thus, the aromatic hydrocarbons could be, in some cases, interacting synergistically.

Many biological and environmental variables affect sensitivity of Alaskan species to the WSF'S of oils. For example, pelagic fish and invertebrates are more sensitive than intertidal species. There can be extreme differences in sensitivity between the life stages of one species. For example, eggs often seem as tolerant as adults to short-term exposures, but abnormalities can appear after the eggs hatch. Salmon (Oncorhynchus spp.) alevins become much more sensitive to oil as they develop and lose their yolk. Crustacean larvae are usually sensitive to WSF'S and are affected within minutes of exposure. In fact, the lowest LC50 (concentration that killed 50% of the test animals) we have measured was 0.2 ppm aromatic hydrocarbons for Stage VI coonstripe shrimp (Pandalus hypsinotus) larvae exposed to Cook Inlet WSF. The effect of temperature on toxicity of hydrocarbons varies for each species and for the hydrocarbons tested. Low temperatures, however, increase the persistence of

hydrocarbons in water. Salinity consistently increases the toxicity of hydrocarbons to salmonids, and juvenile salmonids are about twice as sensitive in seawater as in freshwater.

The rate and quantities of hydrocarbons found in test organisms vary considerably and depend on the compounds tested, the life stages and species of animal tested, and the tissues analyzed. Naphthalene and methylated naphthalene, for example, reach higher concentrations in the test animals than other aromatic hydrocarbons. Concentrations of aromatic hydrocarbons in crustacean larvae usually equilibrate within minutes to concentrations of aromatic hydrocarbons in the test water, whereas salmon eggs required several days. Fish accumulate aromatic hydrocarbons rapidly, whereas blue mussels (Mytilus edulis) accumulate them more slowly

Metabolism can be an important mechanism for ridding tissues of hydrocarbons. Of the animals tested, fish had the greatest ability to metabolize hydrocarbons. For invertebrates, however, metabolism does not appear to be important. Fish and invertebrates usually eliminate low molecular weight aromatic hydrocarbons and their metabolizes via the gills.

Oil and its components have a variety of sublethal effects that can affect population size. Feeding rates of fish and invertebrates are frequently reduced during long-term exposures to crude oil at concentrations that are 15-30% of the short-term LC50; thus, growth and energy available for growth (scope-for-growth or energy budget) are decreased.

Sensitivity of an organism in a laboratory study is different from vulnerability after an oil spill. Laboratory tests isolate one variable at a time, whereas oil spills have many variables operating at the same time, and each spill is unique. Planktonic, larvae are among the most vulnerable organisms after an oil spill because they are sensitive to oil, are affected after only minutes of exposure, and cannot avoid spilled oil. In fact, some larvae are killed within minutes by concentrations of crude oil that are <l ppm. Other animals, like fish, may be nearly as sensitive as planktonic larvae but can probably avoid an oil-contaminated area. Although intertidal animals are not sensitive to oil spills and may require long-term exposures to about 20-25% of their LC50 before growth is affected, many are sessile and must rely solely on physiological tolerance to endure exposure to oil.

Studies at Port Valdez

Effluent from the ballast-water treatment plant at Port Valdez is rapidly diluted, and in the short term, is apparently not toxic. However, concentrations of aromatic hydrocarbons in the effluent were as high as 15 ppm, and dilutions of the effluent as low as 2% (vol/vol) were toxic to some crustacean larvae. Shrimp and fish were less sensitive to the effluent (LC50 of 19-43% dilutions) than larvae. Repeated tests with shrimp and fish suggest that the toxicity of the effluent may be caused by contaminants other than aromatic hydrocarbons.

A decrease in the population of Baltic clams (Macoma balthica) and the presence of hydrocarbons in sediments near the effluent-treatment facility suggest that a continuous discharge of effluent could cause long-term damage.

Drilling Muds

Because drilling muds are rapidly diluted and have low toxicity, they are probably not toxic to planktonic larvae. Unlike the WSF'S of oil, crustacean larvae exposed to drilling muds do not immediately cease swimming or die, and most of the toxicity is apparently caused by physical stress of the particulate in suspension rather than chemical stress. The alkalinity of one mud was quite high, however, and its alkalinity was the primary cause of toxicity.

TABLE OF CONTENTS

INTRODUCTION

This report summarizes all the research on effects of oil on Alaskan organisms conducted by the Auke Bay Laboratory from the first study in 1970 through 1981. Most of the research was conducted in the laboratory. The report includes all the major findings from 62 studies, regardless of funding source.

Emphasis in earlier years was on short-term toxicity of crude oil to fish and invertebrates. More recently, most of the studies have been long term and have determined sublethal effects of crude oil and its components on fish and invertebrates. Several field studies were conducted at the Trans-Alaska Pipeline System terminal at Port Valdez, Alaska, to determine the effects of effluent from the ballast-water treatment plant on the marine environment. We also evaluated the toxicity of drilling muds to several species of crustacean larvae found in Alaska.

We review the research by categories, such as toxicity, uptake and metabolism, and effects of oil and its components on growth, and include abstracts for all 62 studies (Appendix A). Appendix B contains a list of all the species tested, including scientific names. Some of the results of our research have been published in individual reports, some have been included in earlier reviews (Evans and Rice 1974; Karinen 1977; Rice 1977; Rice et al. 1977a), and other results are unpublished. A comprehensive review of all our oil-effect studies, however, has never been included in one document.

Oil-effects research by the Auke Bay Laboratory began after enormous deposits of oil were discovered at Prudhoe Bay on Alaska's Arctic coast. The planning and construction of the Trans-Alaska Pipeline, through which nearly 20% of U.S. oil production now flows, aroused great concern in the fishing industry about the potential for catastrophic effects of oil pollution on fish and their habitat. Until that time, little information was available on effects of oil on Alaskan species under cold-water conditions. Baseline observations in Port Valdez (terminus of the pipeline) began in 1970 with funding from the U.S. Fish and Wildlife Service. The first Laboratory experiments on oil toxicity began in late 1971, and a study of pink salmon (Oncorhynchus gorbuscha) avoidance of oil was completed in 1972. In 1973, the Auke Bay Laboratory Environmental Physiology Section was formed with a staff of three biologists and funding from the National Marine Fisheries Service (NMFS).

A core research staff has been funded by NMFS continuously through 1982 at an annual level of $150,000-$200,000.

The first non-Government funding for toxicity studies at the Laboratory was from Shell Oil Company in 1974. Money from this contract provided basic analytical equipment and an increase in staff. Other contracts followed with the Outer Continental Shelf Environmental Assessment Program (OCSEAP) (1975 to present), Environmental Protection Agency (1979-80), and Office of Marine Pollution Assessment (1979 to 1981).

METHODS

Comparisons of sensitivities of different species or life stages can be misleading if there are differences in types of chemical analyses, exposure methods (static versus flow-through exposure), or response rates of the animals. The complex composition and physical behavior of crude oil test solutions requires sophisticated chemical analyses and complicated exposure apparatus. Standard gravimetric methods for analyzing crude oil are not suitable for determining concentrations of crude oil in water. These methods measure nonvolatile oil and grease by weight after volatile components are extracted; thus, volatile toxic compounds, such as the monoaromatic hydrocarbons, are not measured.

We used infrared (IR) and ultraviolet (UV) spectroscopy, UV fluorescence, and gas chromatography to analyze oil and the WSF'S of oil. Spectrophotometric and fluorimetric methods, however, do not separate or quantify individual compounds. Gas chromatography is the most useful method for detailed analyses of components of crude oil because individual compounds can be separated and measured. Measurement of individual compounds in oil is important because the toxicity, persistence, and degradation of each compound are variable. Furthermore, temperature influences the stability of compounds in test solutions by affecting rates of evaporation and biodegradation.

We report the concentrations of WSF'S in parts per million total monoaromatic and diaromatic hydrocarbons (benzene through methylated naphthalenes). Monoaromatic hydrocarbons are slightly water-soluble and predominate in water-soluble fractions (WSF'S) of crude oil (Short et al. 1976). Other aromatic hydrocarbons are found only in trace quantities in the WSF'S.

Comparisons between static and flow-through tests are not appropriate because hydrocarbons evaporate rapidly from solutions; therefore, static exposures are less toxic than flow-through exposures (Table 1; Brodersen and Rice in preparation). Furthermore, ratios of toxicities from static exposures to toxicities from flow-through exposures vary with the species tested because some species, such as salmon fry, respond rapidly whereas others, such as blue mussels, respond slowly.

Table 1.--Short-term (96-h) toxicity of Cook Inlet crude oil water-soluble fractions in static and flow-through exposures to pink salmon fry and kelp shrimp (Brodersen and Rice in preparation). LC50 is the concentration that killed 50% of the animals.

Species	Static 96-h LC50 (ppm)	Flow-through 96-h LC50 (ppm)
Pink salmon	2.5	1.0
Kelp shrimp	4.0	1.4

Flow-through exposures are superior to static exposures because flow-through exposures have constant concentrations of hydrocarbons, supply oxygen, and remove metabolic wastes. We have built two devices for producing stable concentrations of aromatic hydrocarbons for short- and long-term tests (Moles et al. in press). For tests with individual components of crude oil, a syringe pump injects hydrocarbons into a stream of water at a rate that does not exceed the volubility of the compound. To prepare WSF'S, water is dripped through a 2-m long X 15-cm diameter glass column that has a 15-cm layer of constantly replenished oil at the top. Water flowing through the oil layer absorbs aromatic hydrocarbons and continues through the column into the exposure tanks.

Different species, or even life stages of the same species, respond to oil and its components at different rates; therefore, simple comparisons of sensitivities of animals over a standard time interval (such as a 4-d LC50) are inadequate. For example, salmon fry surviving the first 24 h of exposure to oil are unlikely to succumb to further exposure (Rice 1973; Rice et al. 1975; Moles and Rice in preparation). In contrast, adult coonstripe shrimp

(Pandalus hypsinotus) died each day in a test lasting 28 d (Brodersen and Carls in preparation). Other studies have shown that blue mussels did not die after exposure for 21 d to lethal concentrations but do die after exposure to the same concentrations for 28 d (Stickle et al. in press).

Sublethal or moribund responses are often detected in test organisms well before they die. In these cases, we calculated median effective concentrations (EC50's: concentrations at which 50% of the animals show a particular sublethal response). For example, crustacean larvae are unable to swim after a 20-min exposure to lethal concentrations of WSF but do not die until 7-10 d later (Brodersen in preparation). In nature, these larvae would probably be eaten or buried in sediments; therefore, they may be considered ecologically dead.

TOXICITY OF OILS AND THEIR COMPONENTS

Because oils are a mixture of many different compounds, toxicity tests with crude oil or refined oils require a different experimental approach than tests for other toxic substances, such as metals. Physical properties affect the rate and amount that a given compound in oil is incorporated into water. These properties include, but are not limited to, volubility of the compound, viscosity and composition of the oil, ease of mixing the oil with water, and the time that the compound and water remain mixed.

Once in water, the persistence of oil components is influenced by a variety of processes, such as biodegradation and evaporation, which in turn are affected by temperature. Biodegradation and evaporation are important even at low temperatures that characterize Alaskan waters. Cheatham and Rice (in preparation) demonstrate that losses of total aromatic hydrocarbons from WSF's of crude oil are significant; however, the rate of loss is reduced at lower temperatures (Fig. 1). Diaromatic hydrocarbons are lost primarily through biodegradation, whereas low molecular weight monoaromatic hydrocarbons, which have higher vapor pressures than diaromatic hydrocarbons, are primarily lost through evaporation. For example, at 12*C, up to 80% of the low molecular-weight monoaromatic hydrocarbons are lost from the WSF of Cook Inlet crude oil in static tests lasting 96 h. Oil and seawater mixtures at

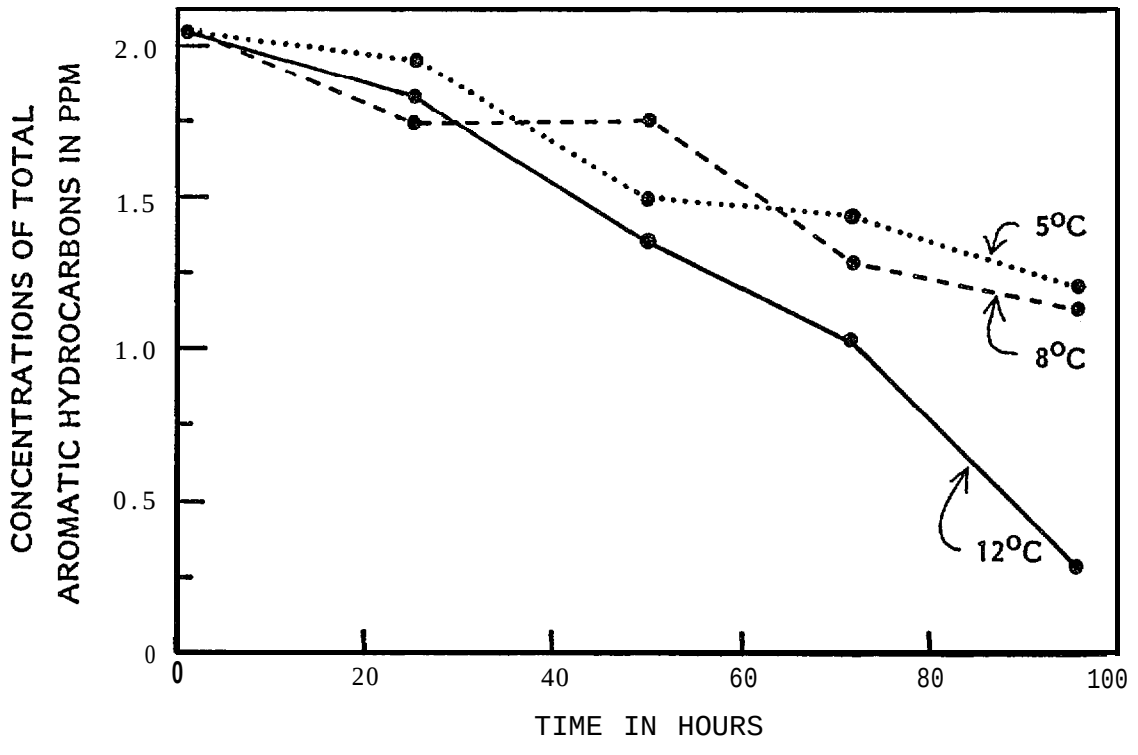

Figure 1. --Concentration of total aromatic hydrocarbons in water-soluble fractions of Cook Inlet crude oil as measured by gas chromatography at 24-h intervals. Solutions were kept at either 5°, 8°, or 12°C and were not aerated (from: Cheatham and Rice in preparation).

low temperatures are more toxic because aromatic hydrocarbons persist longer in cold water than in warm water.

The WSF's of Alaskan crude oils are similar (Table 2), but concentrations of different aromatic hydrocarbons in the WSF's are usually different. For example, the aromatic hydrocarbons in the WSF's of Prudhoe Bay crude oil, Cook Inlet crude oil, and effluent from the ballast-water treatment plant at Port Valdez are nearly identical and have a ratio of monoaromatic hydrocarbons to diaromatic hydrocarbons of about 20:1. The similarity in composition of WSF's of crude oils is influenced by the volubility of the individual aromatic hydrocarbons in water and their ability to be extracted into water. In contrast, the WSF of No. 2 fuel oil has a much lower concentration of monoaromatic hydrocarbons than the WSF of other oils (the ratio of monoaromatic hydrocarbons to diaromatic hydrocarbons is about 1:1) because most monoaromatic hydrocarbons are removed during refining.

Table 2. --Representative concentrations of aromatic hydrocarbons in effluent from the Port Valdez tanker ballast treatment plant and in water-soluble fraction (WSF) of Prudhoe Bay and Cook Inlet crude oil and No. 2 fuel oil.

Component	Concentration (ppm)			
	Prudhoe Bay[a] WSF	Cook Inlet[a] WSF	Ballast[a] effluent	Fuel oil[b] WSF
Benzene	1.8	3.2	3.2	0.11
Toluene	2.0	2.5	2.2	0.17
o-xylene	0.28	0.35	0.32	0.12
m- and p-xylene	0.58	0.78	0.68	0.17
Naphthalene	0.084	0.15	0.098	0.15
1-Methylnaphthalene	0.032	0.066	0.049	0.13
2-Methylnaphthalene	0.048	0.088	0.066	0.25
Ratio of all monoaromatic hydrocarbons to diaromatic hydrocarbons	19.3:1	15.7:1	23.6:1	1.1:1

[a] From Rice et al. 1981.
[b] From Rice et al. 1979.

Although the ratio of monoaromatic to diaromatic hydrocarbons is different for the WSF's of No. 2 fuel oil and Cook Inlet crude oil, different animals have similar patterns of sensitivity to the WSF's of the two oils. For example, pelagic animals sensitive to crude oil are also sensitive to No. 2 fuel oil, and tolerant species are tolerant to both crude oil and No. 2 fuel oil. Number 2 fuel oil, however, is consistently more toxic than Cook Inlet crude oil (Table 3). The LC50'S for No. 2 fuel oil (measured by gas chromatography) are 10-59% of the LC50'S for crude oil, and the LC50'S for fuel oil (measured by IR spectrophotometry) are 18-36% of the LC50's for crude oil.

Although monoaromatic hydrocarbons, which predominate in crude oil WSF, are only 10% as toxic as diaromatic hydrocarbons, such as naphthalene, monoaromatic hydrocarbons are more water soluble and have higher concentrations in the WSF's of crude oil than diaromatic hydrocarbons. The LC50'S of crude oil, measured as total concentration of aromatic hydrocarbons, reflect concentrations of toxic compounds that consist of high percentages of the less toxic

Table 3.--Short-term toxicity of the water-soluble fractions (WSF's) of Cook Inlet crude oil and No. 2 fuel oil to Alaskan marine organisms (Rice et al. 1979). LC50 is the concentration that killed 50% of the test animals.

	96-h LC50 (ppm total aromatic hydrocarbons)	
Organism	Cook Inlet crude oil	No. 2 fuel oil
Salmonids	1.5-1.66	0.97
Benthic fish	3.96->6	1.31->2
Shrimp	0.87-1.86	0.36-1.1
Crab	3.6->10	1.02->3

monoaromatic hydrocarbons. In contrast, the high toxicity of No. 2 fuel oil is probably caused by the low concentrations of very toxic diaromatic hydrocarbons and the low viscosity of No. 2 fuel oil, which permits more diaromatic hydrocarbons to dissolve in water.

The contribution of each class of compounds to the toxicity of the WSF is difficult to isolate because a WSF is a complex mixture of paraffins and aromatic hydrocarbons. Furthermore, the interactions of aromatic hydrocarbons in WSF's appear to be complex and difficult to predict. Aromatic hydrocarbons have been presumed to be major contributors to the toxicity of WSF's because they are present and toxic, but quantitative studies on toxic interactions of aromatic or other compounds are lacking. Kern et al. "(in press) concluded that phenolic compounds are not major contributors to the toxicity of WSF's because their concentrations in WSF's are too low. When a simulated WSF was made using the 10 aromatic hydrocarbons that predominate in the WSF of crude oil (see Table 2), the toxicity of the simulated WSF was only 20-30% of the toxicity of crude oil WSF, which was made by mixing oil into water, even though the proportions of individual aromatic hydrocarbons in both WSF's were the same (Rice and Andrews, unpublished data on file ABL). Other laboratory experiments testing pairs of aromatic hydrocarbons indicate that the toxicities of some aromatic hydrocarbons in the WSF are synergistic (Rice and Andrews, unpublished data on file ABL).

COMPARATIVE ANIMAL SENSITIVITIES

The effects of oil on the marine fauna in Alaska need to be assessed because many species of fish and shellfish support commercial fisheries or are the food sources for these valuable fisheries. Toxicity tests previous to our studies used warm-water species and crude oils not found in Alaska; therefore, the results from these tests cannot be used to predict sensitivities of species and life stages in Alaska. When assessing the potential effect of an oil spill, it is necessary to know whether some groups of animals are more sensitive than others and what factors affect this sensitivity. We have found that a variety of biological and environmental variables affect sensitivity of Alaskan species.

Effect of Habitat Adaptations on Sensitivity to Oil

Different phylogenetic groups and animals living in different habitats have different patterns of sensitivity to oil. In short-term exposures, sensitivity of animals generally increases from lower invertebrates to higher invertebrates to fish (Rice et al. 1976b; Rice et al. 1979). Variation in sensitivities is wide, however, and there are many exceptions. The most distinctive pattern is the correlation between sensitivity of test animals and their habitat (Table 4). Many species have specialized structures and adaptations for survival in different niches and habitats, such as exoskeletons or cryptic behavior. These adaptations can also affect the sensitivity of an animal to oil. Pelagic fish and invertebrates tend to be the most sensitive (LC50's of 1-5 ppm aromatic hydrocarbons), benthic species are moderately

Table 4.--Ranges of sensitivities (96-h LC50 in parts per million aromatic hydrocarbons) for different habitat groups exposed to Cook Inlet WSF (Rice et al. 1979).

	Habitat		
Organism	Pelagic	Benthic	Intertidal
Fish	1-3	4->5	>12
Crab and shrimp	1-5	3-5	8->10
Mollusc	--	4->8	8

sensitive (LC50'S 3->8 ppm aromatic hydrocarbons), and intertidal fish and invertebrates are usually the least sensitive (LC50's of >8 ppm) (Table 4).

Pelagic animals are the most mobile, have the most uniform environment of the three habitat groups, and are most sensitive to stress. Intertidal animals, which inhabit a highly variable and stressful habitat, have little or no mobility or ability to escape but are well adapted to withstand natural stresses (Taylor and Karinen 1977; Rice et al. 1979). Because intertidal animals can withstand natural stresses, they also resist stress caused by petroleum hydrocarbons.

Sensitivity of Different Life Stages to Oil

Sensitivities to oil are different for each life stage of a species and for the same life stages of different species. Extrapolation of the sensitivity of eggs or larvae to other untested species and groups is, therefore, not warranted. Extreme tolerances and extreme sensitivities have been found in the early life stages of fish and invertebrates. For example, salmon eggs and alevins are more tolerant to short-term (96-h) exposures of petroleum hydrocarbons than fry or juvenile salmon. Eggs of pink salmon and coho salmon (Oncorhynchus kisutch) exposed to benzene had 96-h LC50's of 340 ppm and 540 ppm, respectively; whereas, pink salmon and coho salmon fry had LC50's of 15.3 ppm and 9.8 ppm, respectively (Moles et al. 1979; Fig. 2).

Salmon eggs are more sensitive to long-term exposures than short-term exposures for at least two reasons: 1) Hydrocarbons move slowly across egg membranes (Kern and Rice 1981), and 2) large quantities of hydrocarbons are accumulated and sequestered in the lipid-rich yolk rather than in developing tissues. Thirty percent of coho salmon eggs exposed to toluene and 60% of those exposed to naphthalene for 17 d died on hatching even though the exposure levels (1.8 ppm toluene and 0.11 ppm naphthalene) were <1% of the 96-h LC50 for eggs (Kern and Rice 1981).

The early life stages of most salmon are spent in freshwater; however, pink salmon often deposit their eggs in the intertidal reaches of streams, and salinity can modify the sensitivity of the early-life stages of this species. For example, Moles et al. (in preparation) found that at WSF concentrations of

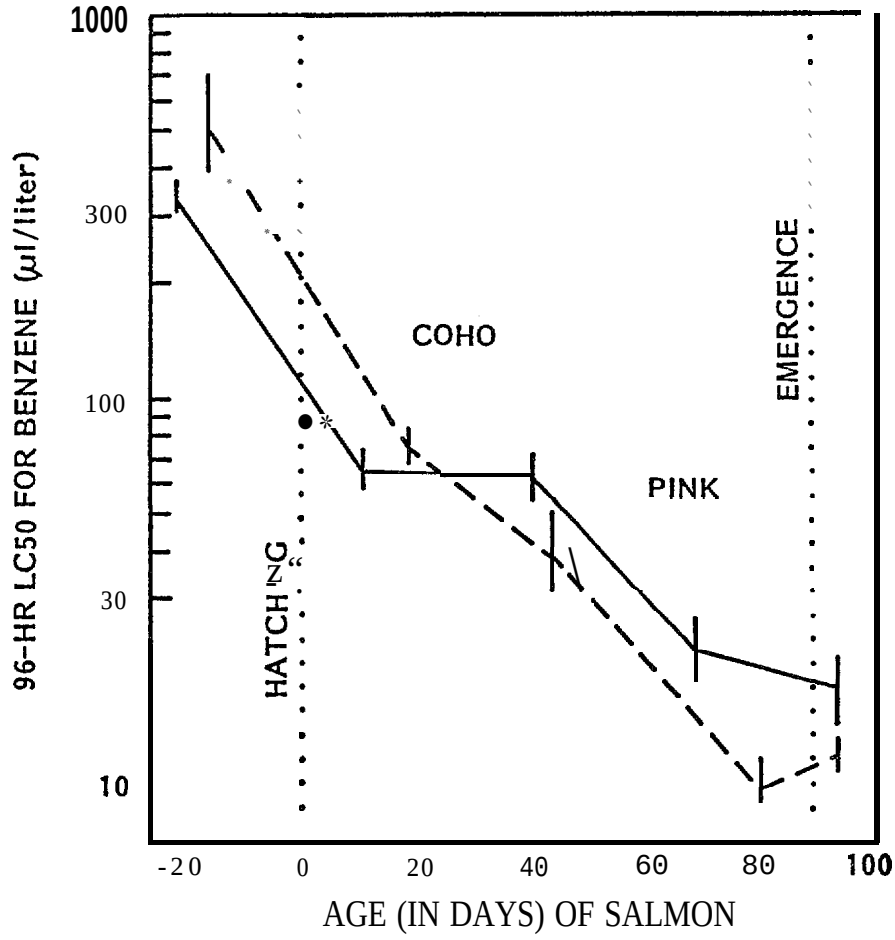

Figure 2. --Sensitivity (96-h LC50's) of early life stages of pink salmon and coho salmon to benzene. Vertical bars indicate 95% confidence intervals (Moles et al. 1979).

2.4 ppm aromatic hydrocarbons, pink salmon eggs in fresh water died in 16 d, whereas eggs in intermittent seawater died within 9 d.

Carls and Rice (in preparation) exposed walleye pollock (Theragra chalcogramma) eggs and larvae to the WSF of Cook Inlet crude oil. Eggs exposed for 20 d to concentrations of 2.9 ppm WSF were abnormal, and larvae were affected by even lower concentrations than eggs. The LC50 for larvae, based on initial concentrations, was approximately 1.3 ppm aromatic hydrocarbons.

Shrimp embryos were more tolerant to the WSF of oil than were adults, even in exposures lasting 30 d (Table 5), but eggs are carried by the female throughout development and cannot survive if she does not. Brodersen and Carls (in preparation) exposed gravid coonstripe shrimp and kelp shrimp (Eualus

suckleyi) to several concentrations of crude oil WSF for 28 d (flow-through exposures). In both species, the eggs survived if the female did. Even female shrimp that were weak and apparently near death released swimming larvae indistinguishable from the larvae of control shrimp.

Table 5.--Sensitivities (LC50'S, parts per million aromatic hydrocarbons) of coonstripe shrimp exposed to Cook Inlet crude oil WSF for 96 h and 28 d (Brodersen and Carls in preparation).

Life stage	LC50 in ppm	
	96-h	28-d
Egg	>1.4	>().5
Larva (Stage I-III)	1.0	1.0
Adult	1.4	0.5

Crustacean larvae are generally more sensitive to crude oil WSF than the adults (Brodersen et al. 1977). For four species of adult shrimp and juvenile king crab (Paralithodes camtschatica), 96-h LC50's (static exposures) were between 1.9 ppm and 4.3 ppm of oil in the WSF (measured by infrared spectrophotometry). The LC50's for the Stage I larvae of the same species were between 0.95 ppm and 1.8 ppm of oil. Differences between sensitivities of early- and late-stage larvae can be large. For example, Stage I (96-h) larvae of coonstripe shrimp had an LC50 of 0.2 ppm, whereas Stage VI larvae had a 96-h LC50 of 1.8 ppm (Brodersen et al. 1977). Molting could be impaired during exposure to crude oil (Karinen and Rice 1974; Mecklenburg et al. 1977).

Larval coonstripe shrimp take up hydrocarbons extremely fast: in <10 rein, tissue concentrations of hydrocarbons can reach several times the concentration of hydrocarbons in the water column (Short et al. in preparation). Rapid uptake of hydrocarbons results in a rapid response for larval shrimp and crabs. Larvae in lethal concentrations of hydrocarbons stop swimming in <20 rein, but they may live for 10 d before they die, at least in the laboratory (Brodersen in preparation). In the marine environment, however, planktonic larvae that fail to swim probably die.

Brief exposures to oil WSF are almost as toxic to shrimp larvae as long exposures (Brodersen and Carls in preparation). Both 24-h and 28-d flow-

through LC50'S for coonstripe shrimp larvae were 1 ppm aromatic hydrocarbons (Table 5).

Effect of Temperature on Sensitivity to Oil

The low temperature of Alaskan waters has two effects on the toxicity of aromatic hydrocarbons, effects that are difficult to separate. Low temperature increases the toxicity of oil by increasing the persistence of aromatic hydrocarbons in water and modifies the physiological sensitivity of test animals (Rice et al. 1977a). The LC50'S for fish and invertebrates from cold environments are generally lower than the LC50's for similar species from warmer climates (Rice et al. 1977a). Although differences in LC50's are not large, they are consistent and probably related more to differences in persistence of toxicants at different exposure temperatures than to differences in sensitivities of the species.

The effects of temperature on the sensitivity of Alaskan species are quite variable. For example, in tests where persistence of the toxicant was not a factor, sensitivity of kelp shrimp increases with higher temperature, but pink salmon fry are more sensitive at lower temperatures (Kern et al. 1979; Table 6). In another test with five circumpolar species of fish and amphipods, there was no general relationship between sensitivity to naphthalene and exposure at temperatures of 1° and 10"C (Carls and Kern in press). Like intertidal animals, these circumpolar species are adapted to a very harsh environment and are tolerant to temperatures far higher than those they usually encounter. Unpredictability of the effect of temperature is probably caused by the inseparable effects of physiological response of animals at each temperature and persistence of the toxicants.

Table 6.--Sensitivities (96-h LC50's, parts per million aromatic hydrocarbons) of pink salmon fry and kelp shrimp exposed to Cook Inlet WSF at three temperatures (Kern et al. 1979).

	Crude oil			Toluene		
Species	4°C	8°C	12°c	4°C	8°C	12°C
Pink salmon	1.5	1.7	1.8	6.4	7.6	8.1
Kelp shrimp	1.7	1.9	1.6	21.4	20.2	14.7

Effect of Salinity on Sensitivity to Oil

Several salmonids are twice as sensitive to oil and aromatic hydrocarbons in seawater as they are to the same compounds in fresh water. Dolly Varden (Salvel inus malma), sockeye salmon (Oncorhynchus nerka), and pink salmon fry, for example, are more sensitive to benzene or the WSF of Prudhoe Bay crude oil in seawater (30°/$_{oo}$) than to these compounds in fresh water (Table 7; Moles et al. 1979). The changes in sensitivity were caused by the fish being in seawater rather than differences in composition of the oil in fresh water and seawater. The LC50'S of coho salmon smelts exposed to toluene and naphthalene in water with 0, 10, 20, or 30°/$_{oo}$ salinity increased linearly with salinity, and acclimation to seawater for 12, 22, and 42 d did not change the sensitivity (Stickle et al. 1982).

Table 7.--Sensitivity of three salmonids to the water-soluble fraction (WSF) of Prudhoe Bay crude oil and aromatic hydrocarbons at different salinities.

Species	Hydrocarbon	0°/00	30°/00
Sockeye salmon[a]	WSF	2.2	1.0
	Benzene	10.8	5.6
Pink salmon[a]	WSF	8.0	3.7
	Benzene	17.1	8.5
Coho salmon[b]	Toluene	8.7	4.7
	Naphthalene	1.6	0.9

[a] From Moles et al. 1979.
[b] From Stickle et al. 1982.

Accumulation and metabolism of aromatic hydrocarbons are probably affected by the physiological adjustments that some fish make after moving from fresh water to seawater. For example, Dolly Varden consistently accumulate higher concentrations of toluene and naphthalene (and lower percentages of metabolizes) in their tissues when exposed in seawater than when exposed in fresh water (Thomas and Rice 1981).

Diseased or parasitized organisms are more susceptible to pollutant stress than healthy organisms. Conversely, an organism that has been weakened by exposure to a toxicant is more susceptible to disease. Moles (1980) experimentally controlled the level of parasitism of larval freshwater mussel Anodonta oregonensis on coho salmon fry. Sensitivity of the fry to toluene, naphthalene, and the WSF of crude oil increased as the number of parasites on each fry increased (Fig. 3). Parasitized fry are probably more sensitive to

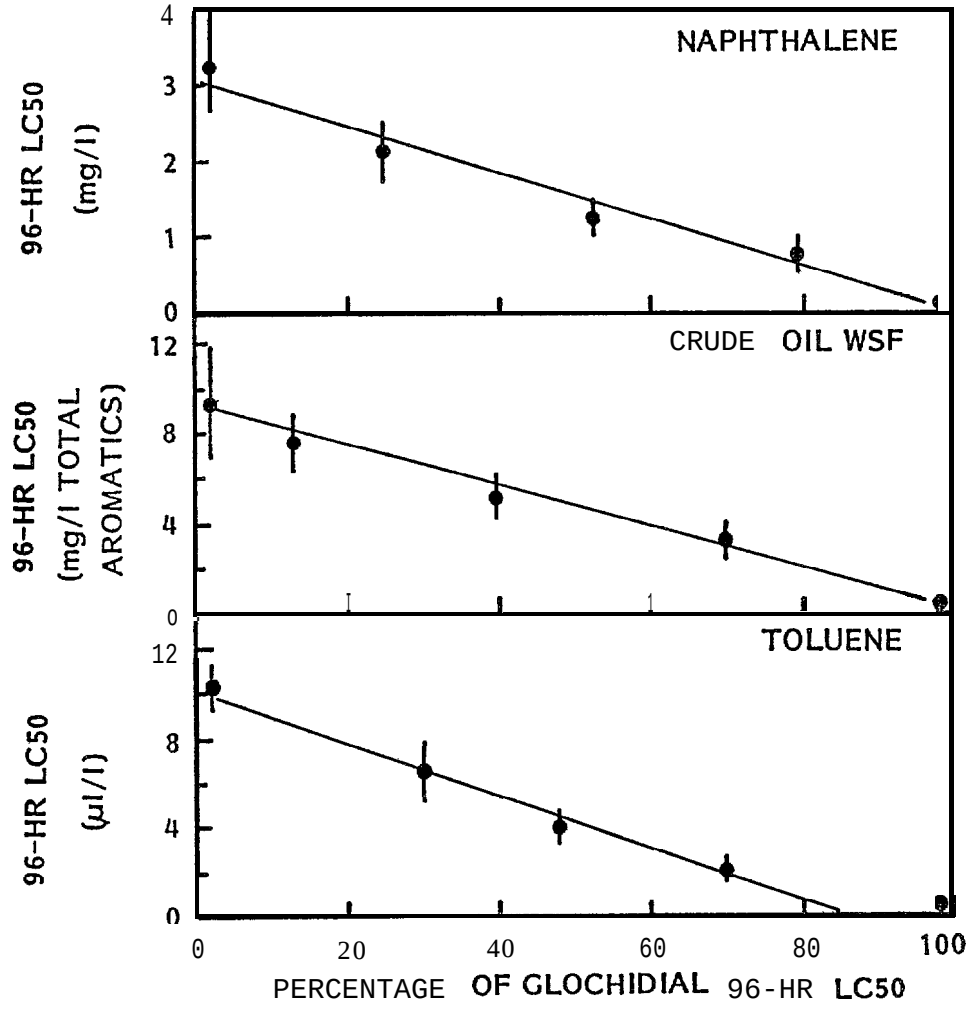

Figure 3.--The effect of infestation by Anodonta oregonensis glochidia on coho salmon fry sensitivity to naphthalene, the water-soluble fraction (WSF) of Prudhoe Bay crude oil, and toluene. The number of glochidia per fish is expressed as a percentage of the glochidial 96-h LC50. The 96-h LC50's of glochidia alone were 115 glochidia per fry (Moles 1980).

these toxicants because parasites reduce the amount of energy available to the fish, and the fish use energy for repair of wounds rather than metabolism of toxicants.

HYDROCARBON UPTAKE AND METABOLISM BY ORGANISMS

Concentrations of hydrocarbons that accumulate in animal tissues can vary considerably depending on tissue, life stage of the animal, and species. Furthermore, several physical, biological, and environmental factors can influence the rate of hydrocarbon uptake, the level of accumulation in tissues, distribution of hydrocarbons between organs, and the ability of the organ to depurate hydrocarbons. Differences in tissue concentration of parent hydrocarbon or metabolize often correlate with differences in survival, severity of sublethal effects, or rate of appearance of sublethal effects.

Physical and Biological Factors Affecting Hydrocarbon Uptake

Hydrocarbons can diffuse into the circulatory system from the stomach (food) or integument, but the primary organ is the gills, which have a large surface area exposed to the environment. The structure, molecular weight, polarity, and methylation of petroleum hydrocarbons affect both the volubility of the compound in the WSF and the rate the hydrocarbons are accumulated in and depurated by animal tissues. Aromatic hydrocarbons accumulate in tissues more readily than aliphatic hydrocarbons (Rice et al. 1977b). Larger compounds, such as methylnaphthalenes, accumulate slowly but to higher concentrations and are retained longer in tissues than the smaller, more polar monoaromatic hydrocarbons (Table 8). Because monoaromatic hydrocarbons are more water soluble than polynuclear aromatic hydrocarbons, they cross membranes more easily than polynuclear aromatic hydrocarbons and are not sequestered in the lipid portions of tissues or organelles as tightly as larger aromatic hydrocarbons (Rice et al. 1976a; Rice et al. 1977a; Kern and Rice 1981; Thomas and Rice 1981, 1982; Short and Rice in preparation). For example, early life stages of coho salmon accumulate 2-3 times more 2-methyl naphthalene than naphthalene (Kern and Rice 1981). Snails, crabs, and adult shrimp accumulate 10 times more naphthalene than toluene (Gharrett and Rice in preparation-a).

Table 8.--The effect of size and methyl ation on the accumulation factor of petroleum aromatic hydrocarbons by pink salmon after 3 h of exposure to the WSF of Cook Inlet crude oil. Accumulation factor is the ratio of tissue to water concentration.

Oil component	Accumulation factor
Toluene	3[a]
Naphthalene	64[b]
Methylnaphthalene	139[b]
Dimethylnaphthal ene	198[b]
Trimethylnaphthalene	380[b]

[a]From Kern and Rice 1981.
[b]From Short and Rice in preparation.

Larger hydrocarbons are sequestered into lipid-rich tissues where they are tightly held in the lipid matrix (Lauren and Rice in preparation). Tissues with high lipid content tend to attract hydrocarbons both from the circulatory system and from less lipophilic tissues (Table 9). Liver and gall bladder in fish, digestive gland in invertebrates, and egg yolk typically accumulate hydrocarbons to concentrations that may be 10 times higher than those in muscle, gill, or embryo (Rice et al. 1977b; Thomas and Rice 1982; Kern et al. in preparation; Gharrett and Rice in preparation-b). Purple shore

Table 9.--Effect of tissue type on the accumulation of naphthalene in pink salmon and purple shore crab. Length of exposure (in hours) is in parentheses. Accumulation factor is the ratio of tissue to water concentration.

Species	Tissue	Maximum accumulation factor
Pink salmon[a]	Viscera	180 (72)
	Muscle	160 (72)
Purple shore crab[b]	Digestive gland	104 (12)
	Thoracic ganglion	15 (12)
	Hemolymph	4.2 (12)

[a]From Rice et al. 1977a.
[b]From Lauren and Rice in preparation.

crabs (Hemigrapsus nudus) exposed to naphthalene had virtually no naphthalene in gill tissue; whereas viscera contained 1,200 times the concentration of naphthalene in the exposure water (Lauren and Rice in preparation).

Different species have different rates of hydrocarbon uptake and accumulation because of morphological and physiological differences. Fish have high metabolic rates and accumulate hydrocarbons rapidly; shrimp are intermediate in both factors; and some lower invertebrates, such as pink scallops (Chlamys hericius) and other bivalves, have low metabolic rates, accumulate hydrocarbons slowly, and incompletely depurate hydrocarbons over weeks or months (Fig. 4).

Different life stages of the same species can have different rates of hydrocarbon uptake and concentrations of accumulated hydrocarbons because of differences in anatomy, activity, and lipid content. For example, coho salmon eggs accumulated hydrocarbons very slowly; however, after long exposures,

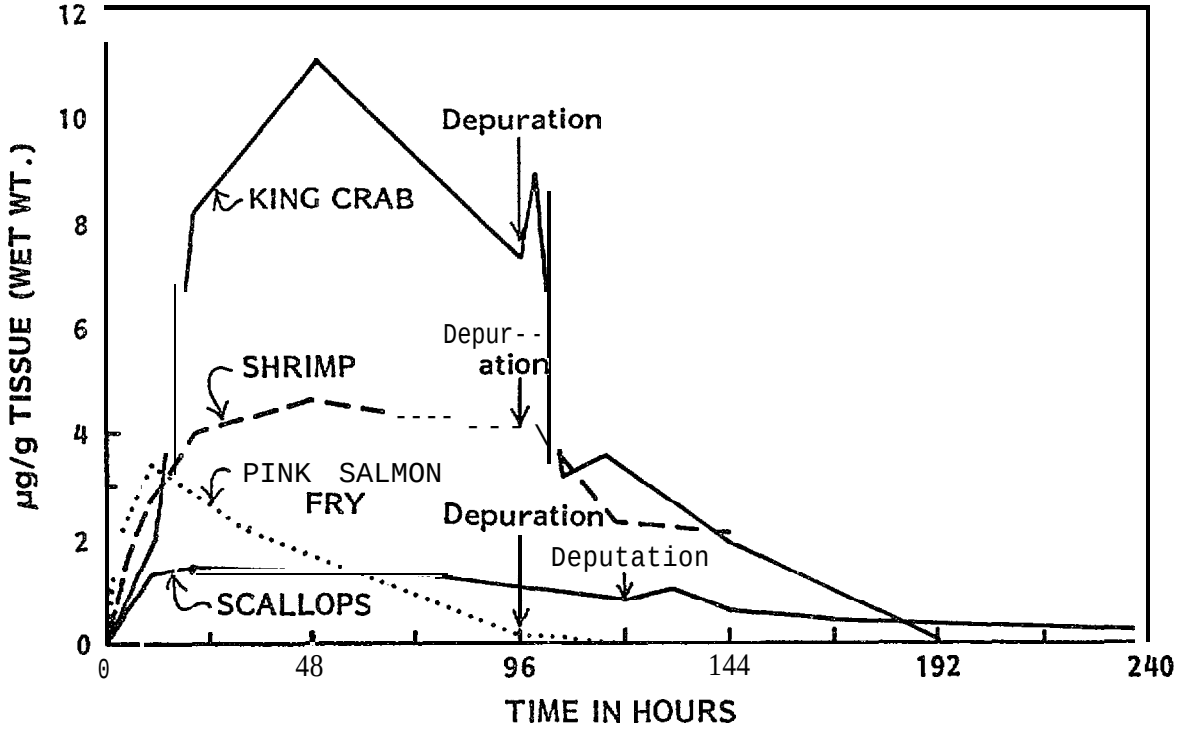

Figure 4.--Concentrations of aromatic hydrocarbons in pink shrimp, pink scallops, pink salmon fry, and king crab during exposure to the water-soluble fraction of Cook Inlet crude oil and during subsequent deputation. Points on the graph represent the sum of all aromatic hydrocarbon concentrations found in groups of five individuals determined at various time intervals (Short and Rice in preparation).

concentrations of accumulated hydrocarbons were high (Kern and Rice 1981). The rates of accumulation and deputation increased as salmon eggs developed into alevins and fry (Fig. 5). The time required for tissue concentrations of hydrocarbons to reach equilibrium with concentrations of hydrocarbons in toxicant baths was over 10 times longer for eggs than for alevins and fry. Salmon fry rapidly absorb hydrocarbons across their gills. Salmon eggs, on the other hand, have a large lipid pool, take up hydrocarbons slowly across the egg membrane, and have higher concentrations of hydrocarbons. Concentrations of aromatic hydrocarbons in shrimp eggs reach equilibrium with concentrations of aromatic hydrocarbons in the toxicant bath in 48 h, and 50% of the total aromatic hydrocarbons are lost from the eggs after 48 h in clean seawater (Kern et al. in preparation). Shrimp larvae accumulate and lose hydrocarbons faster than shrimp eggs. Larval coonstripe shrimp reach equilibrium with naphthalene solutions after only 30 min of exposure, and 70% of the naphthalene is lost 1 h after the shrimp are returned to clean water (Short et al. in preparation).

Processes of Metabolism and Elimination

Hydrocarbons often diffuse from tissues and are slowly extracted from lipid-rich structures and pass to the external environment if the gradient is favorable. Metabolism enhances deputation of hydrocarbons because the metabelites are usually more polar, more water soluble, and, therefore, more easily eliminated from the tissues.

Many fish and some invertebrates, such as crabs, metabolize hydrocarbons (Rice et al. 1977b; Short and Rice in preparation). The specific activity of enzyme systems that metabolize hydrocarbons is much higher in fish than in invertebrates (Lauren and Rice in preparation). In fish, hydrocarbons are primarily metabolized in the liver although some metabolism can occur in many tissues (Thomas and Rice 1981, 1982). In crabs, there is little evidence that hydrocarbons are metabolized in the digestive gland; however, antennal glands have some metabolizing capacity (Lauren and Rice in preparation). Most hydrocarbons are discharged by the gills rather than metabolized by any crab tissue (Lauren and Rice in preparation).

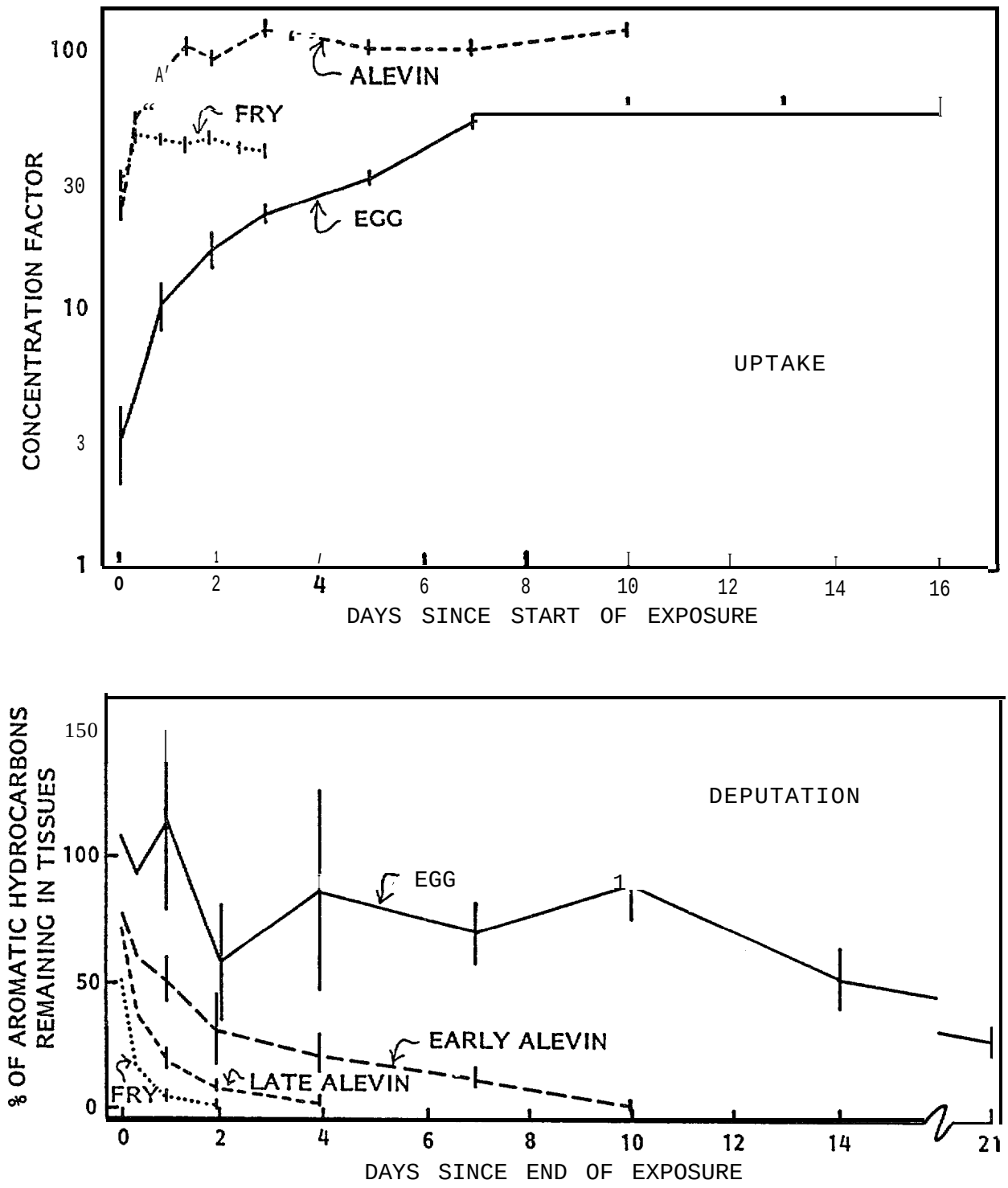

Figure 5.--Accumulation and deputation of naphthalene in early life stages of coho salmon. Fish were exposed to 100 mg/L naphthalene then placed in flowing seawater for deputation (Kern and Rice 1981).

Compounds excreted through the gills, the primary organ for the elimination of many hydrocarbons (Table 10), vary with species. Unmetabolized phenols and other monoaromatic compounds are rapidly eliminated from the gills of fish (Thomas and Rice 1981). Only small amounts of naphthalene and no compounds larger than naphthalene are released from the gills (Thomas and Rice 1982). Gills of crabs are, however, the primary organ for elimination of unmetabolized naphthalenes (Lauren and Rice in preparation). Crabs with blocked nephropores and anus had the same excretion rates as crabs with open nephropores and anus. In crabs, elimination via the urine is unimportant (Lauren and Rice in preparation).

Table 10.--Importance of different excretory pathways of Dolly Varden fed toluene and naphthalene (Thomas and Rice 1981).

Excretory pathway	Carbon-14 excreted during 24 h, as a percentage of administered carbon-14	
	Toluene	Naphthalene
Gill	19.2	12.6
Cloaca	3.4	1.1
Urine	0.008	0.003

Fish slowly excrete high molecular weight hydrocarbons because these compounds are metabolized in the liver and excreted through the bile rather than diffused across gill membranes (Thomas and Rice 1981, 1982). The gall bladder usually has the highest concentration of metabolizes; however, the volume of the gall bladder is, small and seldom accounts for large quantities of metabolizes from mono- and dicyclic aromatic hydrocarbons. Metabolizes of toluene were found in all tissues because these metabolizes are highly mobile and most tissues have some metabolizing capacity. Elimination of metabolizes via the urine in fish (Table 10) is not an important deputation pathway (Thomas and Rice 1981).

Environmental Factors Affecting Hydrocarbon Uptake, Metabolism, and Elimination

Temperature, salinity, and previous exposure to crude oil affect hydrocarbon uptake and deputation by affecting the physiological ability of animals to respond to absorbed hydrocarbons. The effect of temperature is complex, and higher temperatures increase rates of uptake, metabolism, and excretion. At higher temperatures, the rate toluene and naphthalene are accumulated and metabolized in tissues is increased. These compounds are absorbed faster from the stomachs of Dolly Varden at 12°C than at 4"C, and recovery of metabolizes of ^{14}C-labeled naphthalene is always greater at the higher temperature (Table 11) (Thomas and Rice in preparation-a). Decreased sensitivity at higher temperatures probably results from increased metabolism of aromatic hydrocarbons (Thomas and Rice in preparation-a).

Table 11.--Effect of temperature on the recovery of ^{14}C-naphthalene metabolizes 24 h after Dolly Varden (in seawater) were force-fed ^{14}C-naphthalene (Thomas and Rice in preparation-a).

Temperature	Gall bladder	Liver	Brain	Muscle
4°C	66.5	5.1	6.2	17.2
12°C	95.2	15.6	8.6	15.1

Salmonids are much more sensitive to aromatic hydrocarbons in seawater than in fresh water (Moles et al. 1979; Stickle et al. 1982). Higher salinity increases the uptake of hydrocarbons and reduces metabolism of aromatic hydrocarbons. In one experiment, Dolly Varden acclimated to either seawater or fresh water were force-fed toluene or naphthalene. Dolly Varden in seawater absorbed more of these hydrocarbons and had fewer metabolizes than those in fresh water (Table 12; Thomas and Rice in preparation-b). Pink salmon fry exposed to WSF absorbed more hydrocarbons during alternating freshwater and seawater exposures than pink salmon fry exposed to the same oil concentrations in fresh water only (Moles et al. in preparation). Hydrocarbons had greater toxicity when the fry were in seawater because the fry did not metabolize hydrocarbons to more readily excreted forms.

Table 12.--Effect of. salinity on the percentage of ^{14}C-labeled naphthalene recovered as ^{14}C-labeled metabolizes. Dolly Varden were fed ^{14}C-labeled naphthalene at 12°C, and metabolizes were measured 48 h later (Thomas and Rice in preparation-b).

Salinity (°/oo)	Gall bladder	Liver	Brain	Muscle
0	99.9	93.9	99.4	98.9
30	98.0	29.1	13.4	34.9

The rate fish metabolize aromatic hydrocarbons can be increased by exposing fish to sublethal concentrations of hydrocarbons in the aquarium water before force-feeding them ^{14}C-labeled hydrocarbons (Thomas and Rice in press). The rate dietary naphthalene is converted to tissue metabolizes depends on the concentration of naphthalene in the previous exposure (Table 13), the length of the previous exposure, and time lapsed between the previous exposure and subsequent gastric exposure (deputation time). Fish previously exposed for 48 h to naphthalene in the aquarium water had higher concentrations of metabolites in tissues after the second exposure than those that had no previous exposure. A previous exposure of only 24 h, however, did not affect the concentrations of metabolizes in tissues after the fish were fed naphthalene. As deputation time increased, the percentages of carbon-14 recovered in the metabolize fraction from the tissues was greatly decreased. In fact, after

Table 13.--Effect of previous exposure concentration on metabolism of ^{14}C-naphthalene fed intragastrically to Dolly Varden in seawater (Thomas and Rice in preparation-a).

Previous exposure concentration (% 96-h LC50)	Percent of ^{14}C-labeled naphthalene recovered as metabolizes in tissues			
	Gall bladder	Liver	Brain	Muscle
0	82.3	3.0	0.8	2.3
25	84.5	3.2	0.9	2.6
50	86.8	4.5	1.7	5.6
75	87.5	10.1	12.8	34.3

24 h of deputation, concentrations of tissue metabolizes were similar to those in fish with no previous exposure.

SUBLETHAL EFFECTS OF OIL ON FISH AND INVERTEBRATES

Petroleum hydrocarbons have several different sublethal effects, ranging from behavioral (e.g., avoidance of hydrocarbons: Rice 1973) to transitory physiological effects (e.g., increase in respiration: Thomas and Rice 1979) and long-term physiological effects (e.g., decreased growth: Moles and Rice 1983).

Measurable sublethal effects usually appear at about 25% of the LC50; however, LC50's vary widely between species (Table 14). For example, growth

Table 14. --Sublethal effects of the water-soluble fraction (WSF) and individual components of crude oil on Alaskan marine organisms.

Species	Toxicant	Length of exposure (days)	Parameters affected	Percent of LC50 that affected parameters
Pink salmon[a,b] (fry)	WSF	40	Growth	30
	Naphthalene	40	Growth	28
	WSF	4	Opercular rhythm	20
Pink salmon[c] (alevins)	WSF	10	Growth	10
Coho salmon[d]	Toluene	40	Growth	50
	Naphthalene	40	Growth	32
Seastar[e] (Evasterias troschelii)	WSF	28	Feeding rate	28
	WSF	28	Growth	24
File periwinkle (Thais lima) (now called Nucella lima)	WSF	28	Scope for growth	18
Blue mussel[g,h] (adult)	WSF	28	Scope for growth	19
		28	Byssal thread extrusion	15
Blue mussel[i]	WSF	40	Growth, development	61

[a] Moles and Rice 1983.
[b] Thomas and Rice 1979.
[c] Rice et al. 1975.
[d] Moles et al. 1981.
[e] O'Clair and Rice in press.
[f] Stickle et al. 1984.
[g] Stickle et al. in press.
[h] Babcock et al. in preparation.
[i] O'Clair and Rice in preparation.

of coho salmon fry in fresh water and pink salmon fry in seawater was decreased at naphthalene exposures as low as 32% of the 4-d LC50. At about 30% of the 4-d LC50, metabolic rates of pink salmon fry increase (Thomas and Rice 1979). The increase in energy demand during hydrocarbon exposures at ≥30% of the LC50 probably causes energy reserves to be shunted from growth to hydrocarbon metabolism and excretion. Sublethal concentrations that measurably reduce growth range from 10% of the LC50 for pink salmon (Rice et al. 1975) to 61% of the LC50 for blue mussels (O'Clair and Rice in press).

Measurement of energy utilization and partitioning of energy to different biological functions can be used to detect sublethal responses. The energy available for growth (scope-for-growth) is determined from caloric intake less the body-maintenance costs. Scope-for-growth of the carnivorous periwinkle Thais lima declined to zero when these snails were exposed to 18% of the 28-d LC50 (Stickle et al. 1984). Similarly, scope-for-growth in blue mussels was greatly reduced in the latter part of a 28-d experiment at 20% of the LC50 (Stickle et al. in press).

Declining scope-for-growth is the net effect of hydrocarbons on several processes, but the primary cause of reduced scope-for-growth is reduced feeding rates (Stickle et al. 1984, in press). Concentrations that affected feeding rates were much lower than concentrations that affected the other factors (Stickle et al. 1984, in press). A similar pattern was found with the starfish Evasterias troschelii (Fig. 6; O'Clair and Rice in preparation-a). Feeding rates were significantly reduced at 34% of the LC50, whereas gonad and hepatic indices were affected at higher concentrations.

Growth beyond a critical size is important for survival in some organisms such as juvenile salmonids. Fish that are smaller because of sublethal exposures to petroleum hydrocarbons (Fig. 7) would likely suffer increased predation, and recruitment into the fishery would be reduced (Rice et al. 1975).

Stress caused by exposure to sublethal concentrations of hydrocarbons also changes respiration and metabolic rates. The breathing rate of pink salmon fry exposed to the WSF of Prudhoe Bay crude oil increased at approximately 20% of the 96-h LC50 (Thomas and Rice 1975) and was matched by similar increases in oxygen consumption (Thomas and Rice 1979). Both breathing rate and oxygen consumption of pink salmon fry depend on the concentration of toxicant and reflect increased energy demands resulting from increased tissue concentrations of toxicants.

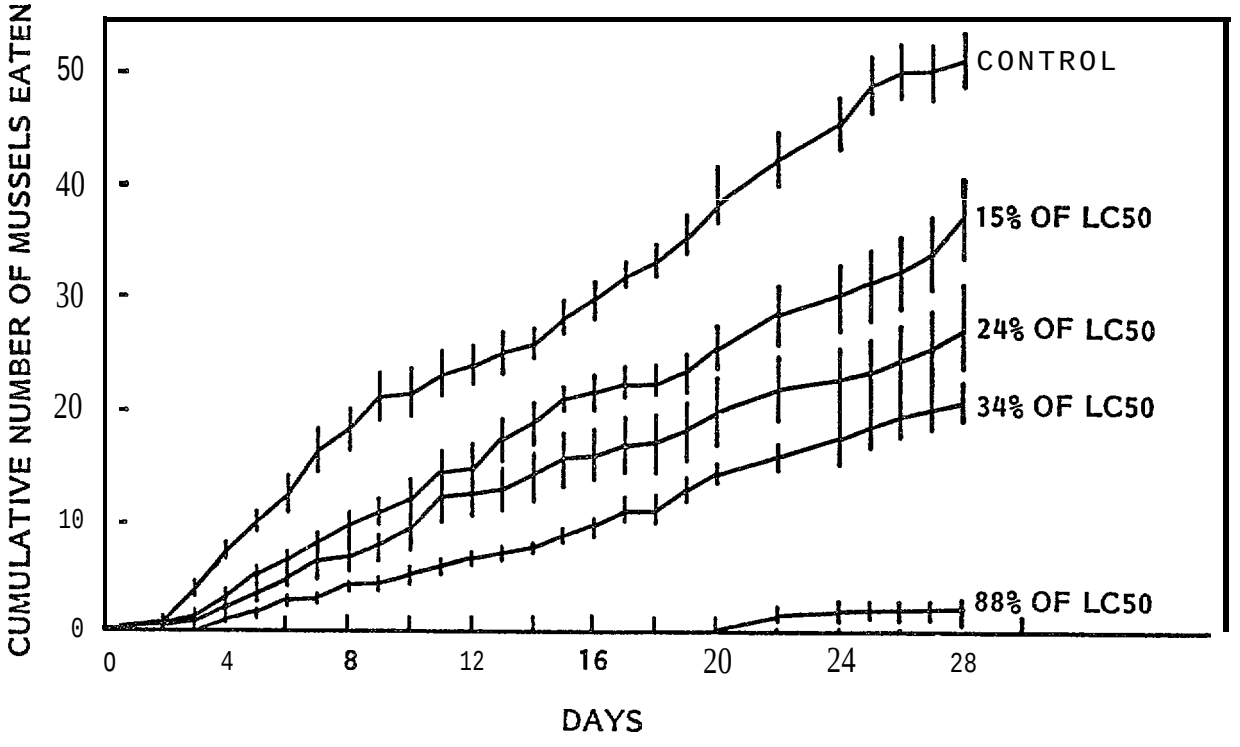

Figure 6.--Cumulative number of mussels eaten by Evasterias troschelii exposed to four concentrations of the water-soluble fraction of Cook Inlet crude oil. Numbers to the right of the curves are percentages of the LC50 for Evasterias troschelii exposed to the water-soluble fraction of Cook Inlet crude oil. Error bars are one standard error of the mean (O'Clair and Rice in press).

Unlike fish, some invertebrates have decreased vital functions when exposed to petroleum hydrocarbons. For example, king crabs exposed to benzene, naphthalene, and the WSF of Cook Inlet crude oil have decreased oxygen consumption and reduced heart rates (Mecklenburg et al. in preparation).

Changes in behavior can be used to detect effects of sublethal concentrations of hydrocarbon. For example, crustacean larvae are unable to swim after a 20-min exposure to WSF but may not die for many days (Brodersen in preparation). Mobile species may avoid petroleum hydrocarbons. Pink salmon fry detected and avoided very low concentrations of Prudhoe Bay crude oil in laboratory conditions (Rice 1973); however, in the natural environment, some fish, such as salmon, may be genetically motivated to migrate along a specific path, even if the path goes through a polluted area. Furthermore, pink salmon fry exposed to benzene had damaged olfactory epitheliums (Babcock in press).

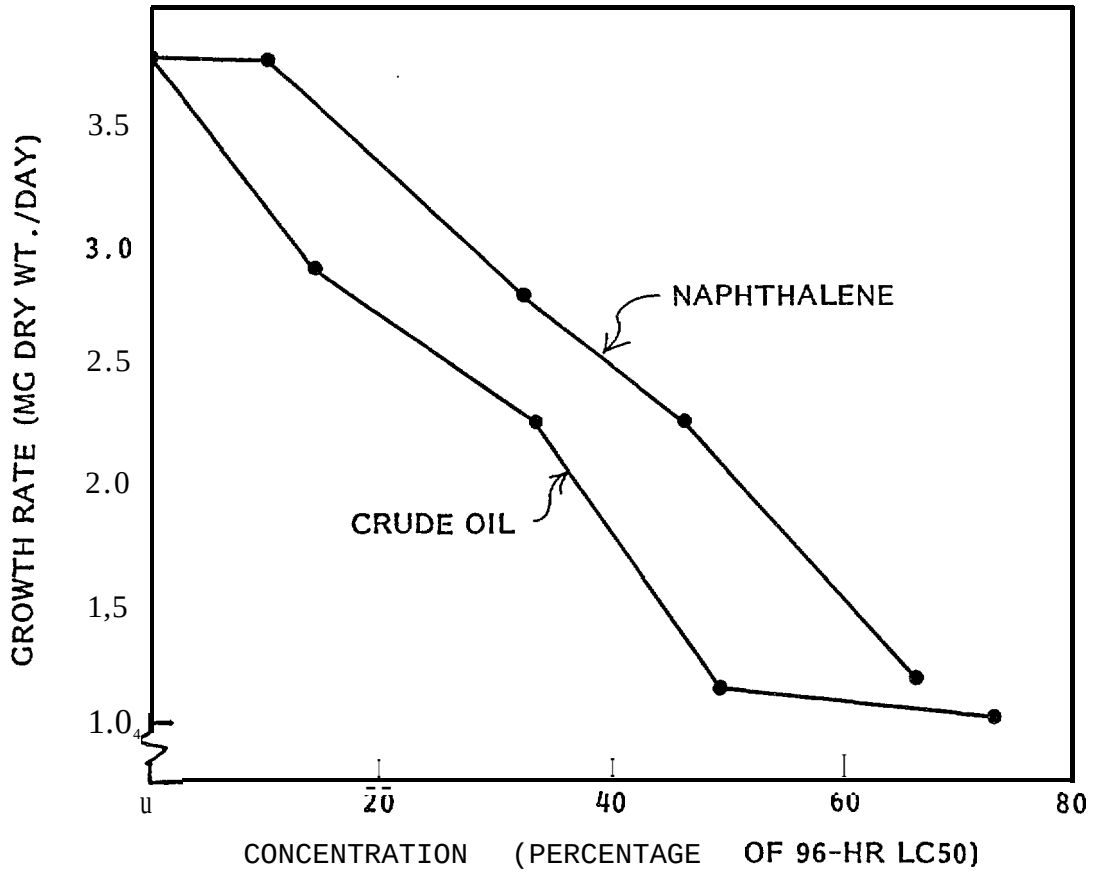

Figure 7. --Growth of pink salmon juveniles per day during a 40-d exposure to concentrations of naphthalene and the water-soluble fraction of Cook Inlet crude oil. Concentrations are expressed as a percentage of the 96-h LC50 (Moles and Rice 1983).

The rate at which blue mussels produce byssal threads is immediately reduced after they are exposed to sublethal concentrations of hydrocarbons (Table 15; Faris et al. in preparation). Byssal thread production, however, has only limited value as a monitoring tool because affected animals start extruding byssal threads immediately after they are returned to clean water. The rate byssal threads are produced thus reflects only current concentrations of hydrocarbons (Babcock et al. in preparation).

Low levels of crude-oil contamination reduce fish and invertebrate populations by reducing reproduction either by causing malformed gonads and gametes or by decreasing the energy available to the organism to contribute to growth or gamete production. Reproduction, like other growth processes, would

Table 15.--Cumulative number of byssal threads produced by blue mussels exposed to the water-soluble fraction of Cook Inlet crude oil (Faris et al. in preparation).

Exposure concentration	Number of threads			
	24 h	48 h	72 h	96 h
Control	16	24	29	37
0.19 ppm	12	19	23	27
0.63 ppm	3	9	15	18
2.34 ppm	0	1	5	12

be affected at hydrocarbon concentrations well below those that affect long-term survival of the animals. At the Auke Bay Laboratory, we are currently determining the effects of petroleum hydrocarbons on the maturation success of gametes in crab and shrimp and the effects of these compounds on the transition of male shrimp into female shrimp.

In conclusion, long-term exposures (30-40 d) to sublethal concentrations of petroleum hydrocarbons (20-30% of the long-term LC50) affect the physiology of most species, reduce growth and reproduction, and change behavior. Exposures longer than 30-40 d to concentrations lower than 20-30% of the long-term LC50 may also have deleterious effects.

STUDIES OF TREATED BALLAST WATER AT PORT VALDEZ

Ballast water containing crude oil is pumped ashore from tankers at Port Valdez where it is treated to remove hydrocarbons before being discharged into Port Valdez. The treatment process is extremely effective and removes more than 99% of the hydrocarbons, but because of the enormous volume of effluent (10-20 million gal/d [38-75 million L/d]), substantial quantities of hydrocarbons are discharged into Port Valdez. The effluent contains 8-15 ppm oil, mostly aromatic hydrocarbons. Research to determine the effects of the effluent on the marine environment of Port Valdez and Prince William Sound consisted of several separate but related studies.

Onsite Laboratory Tests of Treated Tanker-Ballast Water

The toxicity of effluent to several species of fish and invertebrates was studied in a mobile laboratory at the Port Valdez tanker terminal (Rice et al. 1981). Continuous samples of treated ballast water were obtained for toxicity tests by tapping directly into the pipe discharging effluent from the treatment plant. Different larval stages of king crab, Dungeness crab (Cancer magister), coonstripe shrimp, and Pacific herring (Clupea harengus pallasi) were used for the tests, as well as pink salmon fry and adult kelp shrimp.

During the tests, the concentrations of aromatic hydrocarbons in the effluent varied considerably daily; however, they generally declined from >15 ppm to <2 ppm between April and July 1980 (Fig. 8).

Larval stages of shrimp and crab were more sensitive to the effluent than juveniles or adults. All larvae tested in 96-h static tests had LC50's at concentrations between 2% and 26% dilution of the effluent, and affected larvae ceased swimming within 10 min of exposure.

The LC50's for pink salmon fry and adult kelp shrimp in repeated, continuous-flow 48-h and 96-h tests were consistently at 19-43% dilutions of the effluent (Fig. 8). Although the concentration of aromatic hydrocarbons decreased during the study, the toxicity of the effluent remained fairly constant. The 8-h LC50 did not change (Fig. 8); therefore, contaminants other than aromatic hydrocarbons contributed to the toxicity of the effluent. Oxidation products of aromatic hydrocarbons, heavy metals, or hydrogen sulfide are likely possibilities.

In Situ Studies: Survival of Caged Animals in Port Valdez

Survival of caged animals was determined at various distances from the treated ballast-water diffuser in Port Valdez (Karinen et al. in prep.-a). Blue mussels, pink salmon fry, and kelp shrimp were suspended in cages 50 m from the surface and 2 m off the bottom at sites either in the plume of effluent extending from the diffuser or at two control sites. All animals were exposed for 8 d; some blue mussels were exposed for 30 and 90 d. Tissues from test animals were sampled to determine hydrocarbon concentrations.

Less than 10% of the animals died, and their deaths were not attributable to hydrocarbons. After 8 d, low concentrations of aromatic hydrocarbons were detected only in mussels at the two stations nearest the diffuser. Apparently,

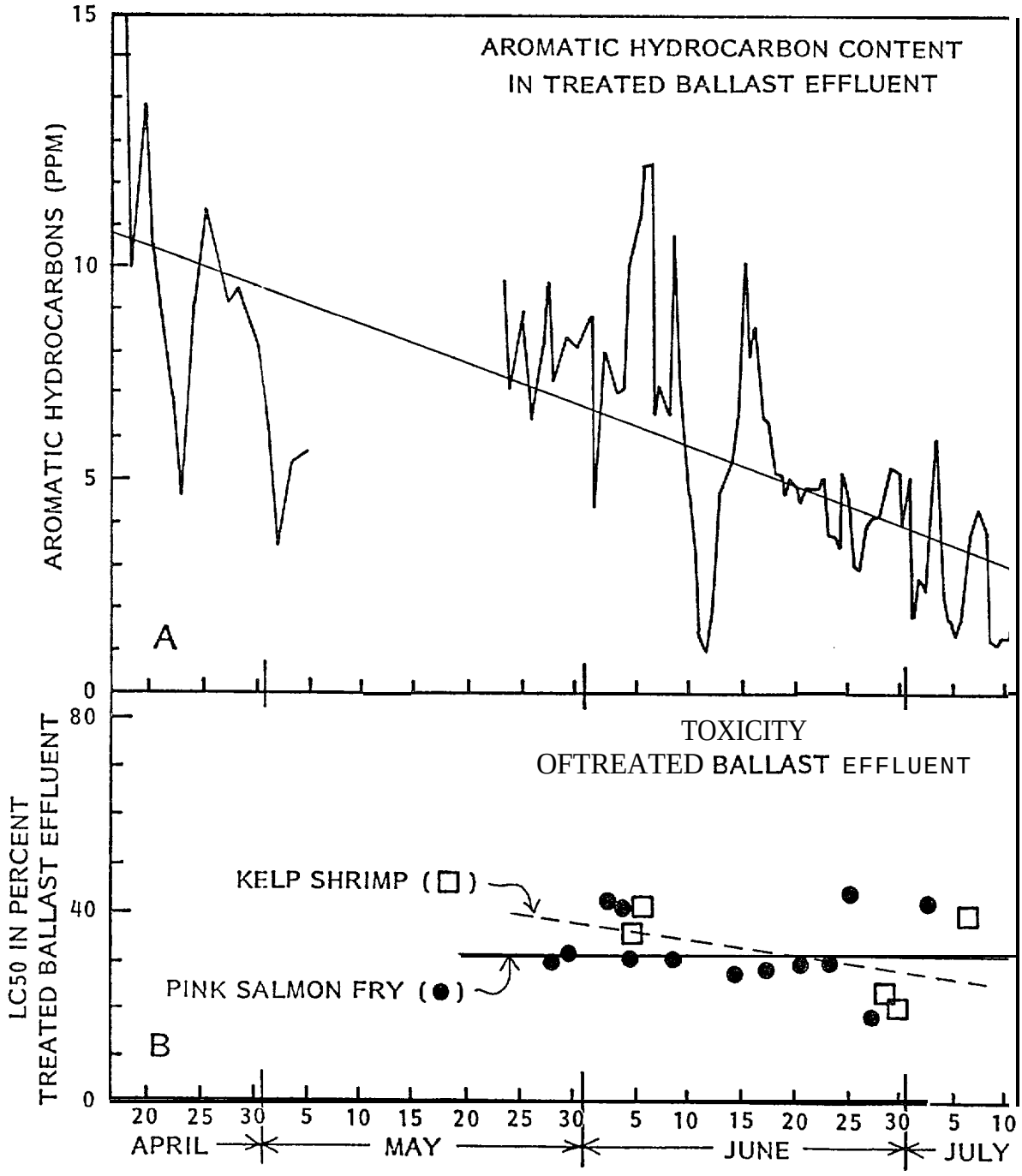

Figure 8.--(A) Daily concentration of aromatic hydrocarbons (monoaromatic and diaromatic) in the effluent from the ballast-water treatment plant at Port Valdez (17 April through 11 July 1980) as determined by gas chromatography. (B) Toxicity of treated ballast water to kelp shrimp and pink salmon fry. Tests were repeated periodically, and 96-h LC50'S are expressed as a percent dilution of the treated ballast water (Rice et al. 1981.)

the effluent is diluted at middepths as it extends horizontally from the diffuser.

Hydrocarbons in the Intertidal Environment of Port Valdez

Hydrocarbon concentrations in sediment, water, and tissues of blue mussels and yellowfin sole (Limanda aspera) were monitored annually from 1977 to 1980 (Karinen et al. in preparation-b). Two sites in Port Valdez and eight sites in Prince William Sound were sampled. Intertidal sediments at four sites (Mineral Creek, Dayville Flats, Constantine Harbor, and Rocky Bay) contained detectable but low concentrations of polyaromatic hydrocarbons; however, there is no conclusive evidence that the treated ballast water was the source of hydrocarbons at any of the sites.

Long-Term Monitoring of Intertidal Clam Populations in Port Valdez

The population of Baltic clams on the Dayville Flats, a mud flat 4 km from the tanker terminal, was measured quantitatively for 12 yr, 1971-82 (Myren and Pella 1977; Myren and Perkins in preparation). During the first 9 yr of the study, 6 yr before and 3 yr after the tanker terminal began operation, the Baltic clam population was remarkably constant. During the most recent 3 yr, however, the number of Baltic clams declined (Fig. 9).

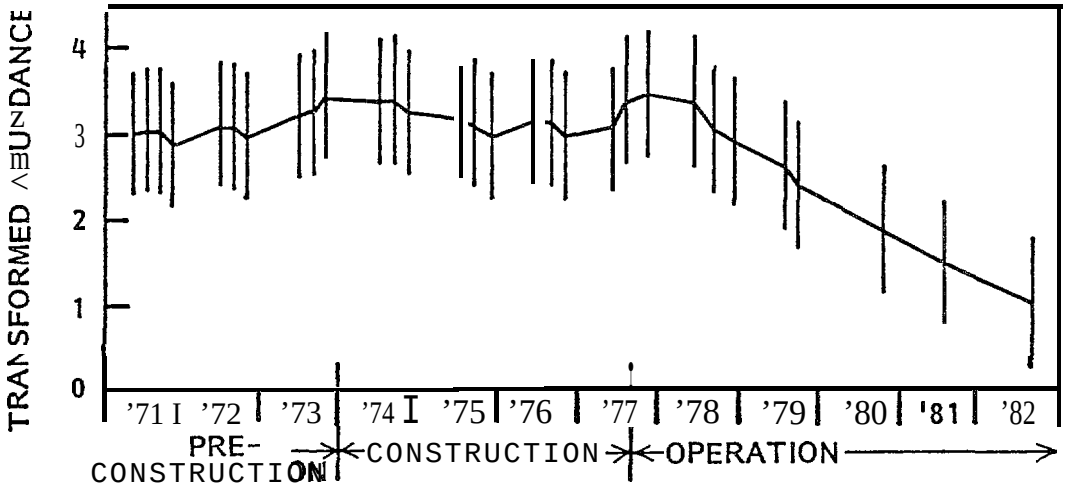

Figure 9. --Abundance of large Baltic clams from the Dayville mudflats of Port Valdez (average of square-root transformed numbers per 100 cm^2 sample area) (Myren and Pella 1977; Myren and Perkins in preparation).

A slight increase in the concentrations of aromatic hydrocarbons in sediments at Dayville Flats (Karinen et al. in preparation-b) has been measured coincident with the decline in the Baltic clam population. The increase in aromatic hydrocarbons is, therefore, suspected to be associated with the decrease in Baltic clam population.

TOXICITY OF DRILLING MUDS

The mud used during drilling of oil wells is a potential source of pollution because large quantities of drilling muds are discharged into the ocean from offshore drilling rigs. Carls and Rice (1984) determined the tolerances of Stage I planktonic larvae of three shrimp and three crab species to six water-based drilling muds. Five of these muds contained ferrochrome lignosulfonate. Because toxicity of drilling muds is a combination of physical and chemical toxicities, the toxicity of some of its components (the WSF of the muds, ferrochrome lignosulfonate, and the particulate barite and bentonite) was also determined.

The toxicity of the drilling muds depends on the composition of the mud and the species tested, and LC50's ranged from 0.9% to 38% (vol/vol). The mud in which particulate remained suspended throughout the test period (Cook Inlet mud) was the most toxic. In general, particulate quickly settled out of suspension, and toxicity was usually low because larvae were only briefly exposed to physical stress. The WSF's of the muds are toxic but much less toxic than whole muds (Table 16), and ferrochrome lignosulfonate accounts for most (46-100%) of the toxicity of the WSF. The toxicity of barite and benton-

Table 16.--Tolerance of shrimp and crab larvae to suspensions and water-soluble fractions (WSF) of Cook Inlet mud (Carls and Rice 1984).

	144-h LC50 (% vol/vol)	
Species	Complete mud	Mud WSF
King crab	0.48	3.34
Dungeness crab	0.20	1.41
Kelp shrimp	0.44	0.47
Dock shrimp	0.05	0.3

ite is low, and alkalinity of only one mud was beyond the pH tolerance limits of the larvae.

Mud solutions inhibit larval swimming only after 1-2 d of exposure; thus, the toxicity of drilling muds is probably more physical than chemical (Fig. lo). In contrast, petroleum hydrocarbons inhibit larval swimming of the same species within minutes after exposure (Rice et al. 1981). Drilling muds contaminated with petroleum hydrocarbons from oil-bearing formations might be more toxic than the muds we tested. Conversely, small quantities of hydrocarbons could be adsorbed on mud particulate and would not be as toxic as comparable concentrations of hydrocarbons in the WSF of crude oil.

Because the toxicity of drilling muds to crustacean larvae is primarily physical, drilling muds discharged into the marine environment probably would not damage planktonic and nektonic communities under most conditions. Toxic

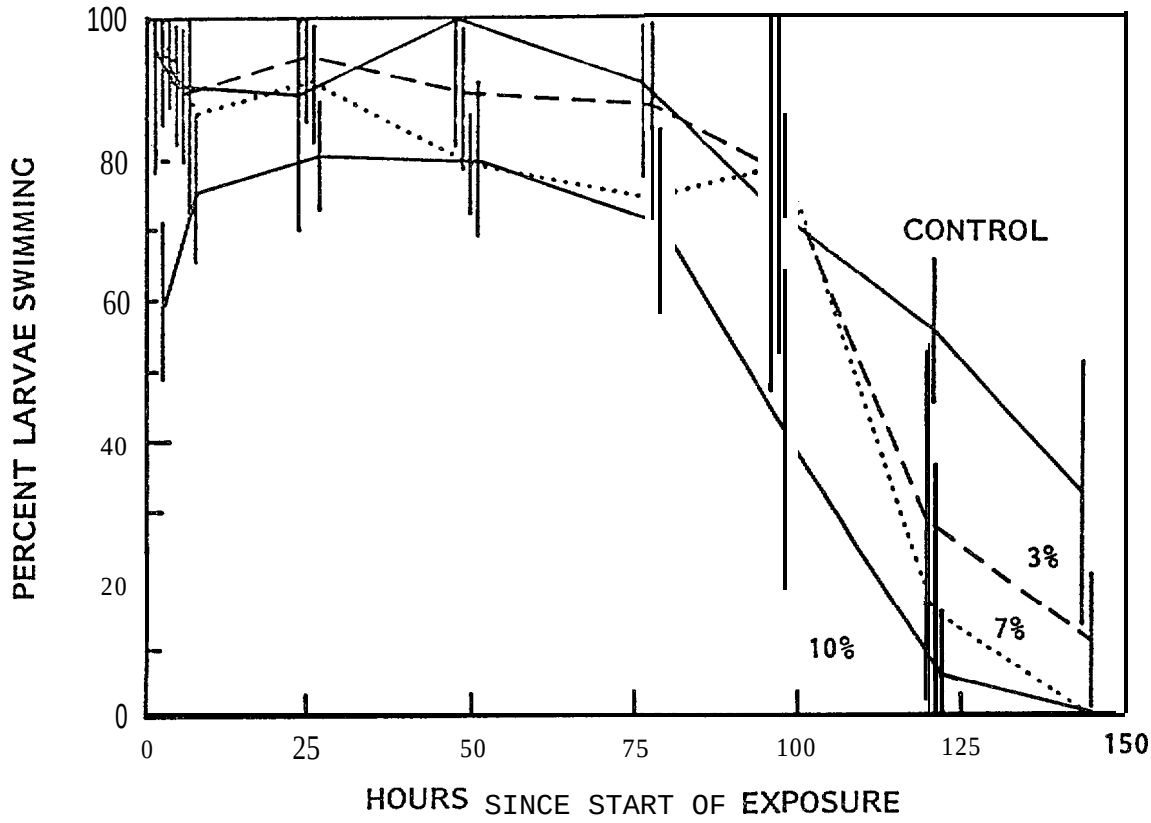

Figure 10.--Inhibition of swimming for larval king crab exposed to different concentrations of used Homer drilling mud. Vertical bars indicate the 95% confidence intervals for the sample means (Carls and Rice 1984).

concentrations of drilling muds are only briefly in the water column and are limited to the immediate area of discharge.

ECOLOGICAL IMPLICATIONS OF THE STUDIES

What will happen if oil is spilled into the marine environment? This question is frequently asked by managers but is seldom answered because the answer is complex, requires massive funding if the research is to be done at the site of an oil spill, and then, unfortunately, may not be useful because each oil spill is unique.

Laboratory studies are cost effective and have contributed to the data base that supports guesses and predictions. Laboratory studies on the effects of oil have identified species, life stages, and biological processes (e.g., growth and molting) that are sensitive, as well as identified environmental factors that affect the toxicity of oil (e.g., temperature) or the sensitivity of organisms (e.g., salinity).

In laboratory studies, one or more variables are tested and the effects of these variables determined. Some of the effects found in these studies, however, may never be observed after an oil spill. For example, in laboratory experiments sublethal concentrations of oil can decrease growth; however, the same concentrations in an actual oil spill would result in the animals being eaten or cause them to migrate to areas of lower oil concentrations. In either case, reduced growth would not be observed.

Although a test animal is sensitive to aromatic hydrocarbons in oil, it may not be vulnerable to an oil spill. For example, fish are sensitive to oil but can swim away from an oil spill and are not vulnerable. In contrast, shrimp larvae are vulnerable because they are very sensitive to oil and cannot move from a spill area. Intertidal species are less sensitive to oil than shrimp larvae and fish, but if crude oil comes ashore, most cannot move out of their contaminated habitat. Consequently, intertidal species are trapped but could survive through adaptive tolerance.

Some habitats, like some species, are more vulnerable than others to spilled oil. Oil spills offshore are rapidly diluted and dispersed, and no apparent lasting effects can be measured. In contrast, spills within confined bays or spills that wash ashore onto marshes or wetlands can have long-term

effects. Marshes and wetlands may erode or the contamination may last for several years and make the habitat unsuitable. Benthos may accumulate hydrocarbons over many years so that even the ocean bottom is vulnerable (Karinen 1980).

Effects of many interacting variables in nature are not quantitatively known. Some pairs of variables (salinity and concentration of oil or effects of two oil components) have been measured in the laboratory, but the complex interaction of many variables is poorly understood. Generally, we know whether a variable will increase or decrease the toxicity of oil, but not the magnitude, especially when the variable is interacting with other variables.

Oil spills can damage organisms if oil concentrations are high enough and the organisms are quickly affected. We have observed short-term toxicity of oil at about 1-3 ppm, concentrations that would briefly occur after oil is spilled and mixed in confining bays.

Fish and crustacean larvae would most likely be affected by an oil spill. Fish respond quickly and are sensitive to concentrations of oil and its components in the low parts per millions. Crustacean larvae are even more sensitive to oil and its components than fish: EC50's and LC50's are between 0.2 and 0.7 ppm. Furthermore, crustacean larvae are more quickly affected (10-30 rein) by oil exposure than fish and cannot move away from a spill area.

Long-term exposures at about 20% of the short-term LC50 can affect respiration, feeding rate, and growth. If an organism stays in the contaminated area, exposure to 0.1-0.2 ppm crude oil for 30 to 40 d could be harmful, particularly to invertebrates. Oil concentrations at 0.1-0.2 ppm can be expected after an oil spill but are not sustained unless oil is being continuously added to the environment. Longer exposures to even lowerconcentrations of oil could affect growth, maturation, and reproduction; however, the length of time and the oil concentrations required are unknown.

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THOMAS, R. E., and S. D. RICE. In preparation-a. Effect of environmental temperature on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden, Salvelinus malma.

THOMAS, R. E., and S. D. RICE. In preparation-b. Effect of salinity on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma.

APPENDIX A.
ABSTRACTS OF ALL PAPERS ON OIL-EFFECTS RESEARCH AT THE AUKE BAY LABORATORY, 1970-81

Babcock, M. M.

In press. Morphology of olfactory epitheliums of pink salmon, Oncorhynchus gorbuscha, and changes following exposure to benzene: a scanning electron microscopy study. In J. S. Gray and M. E. Christiansen (editors), Marine biology of polar regions and effects of stress on marine organisms. John Wiley & Sons, London.

The pink salmon fishery is the most valuable fishery in Prince William Sound, Alaska (USA), and there is great concern that this resource may be damaged by oil pollution from tankers or discharges from a ballast-water treatment plant near Valdez. Because juvenile pink salmon school along shallow estuarine shorelines of Prince William Sound for several weeks before migrating to oceanic feeding grounds, they are vulnerable to oil pollutants from these sources.

Benzene, a major component of crude oil and the effluent from the treatment plant, is water soluble and relatively toxic to fish. To determine the histopathological effects of benzene on olfactory rosettes of pink salmon, I exposed juveniles to sublethal concentrations of benzene in seawater. Fish were exposed in seawater to 4.3 ppm benzene for 12 days or to one of four concentrations ranging from 0.15 to 4.40 ppm benzene for 29 days. (The concentration that killed half the fish in 96-h was 8.47 ppm).

After the exposures, olfactory rosettes from" the fish were examined with scanning electron microscopy. Rosettes from all fry exposed to benzene had exhausted mucous cells. The olfactory lamellae of fry exposed to ≥0.51 ppm benzene had altered distribution of cilia. Olfactory lamellae of fish exposed to 4.3 ppm benzene for 12 days had patchy losses of cilia; olfactory lamellae of fish exposed for 29 days to concentrations of benzene ≥0.51 ppm had a generalized loss of cilia. These differences in cilia loss may indicate regeneration of cilia or different individual responses to benzene.

Exhausted mucous cells and loss of cilia on the olfactory lamellar surfaces could change circulation of water through the olfactory rosettes or otherwise interfere with normal chemosensory reception and consequently affect homing, traditional migratory patterns, feeding activity, and avoidance of predators.

Babcock, M. M., S. D. Rice, and P. J. Arasmith.

In preparation. Reduced byssal thread extrusion and recovery following long-term exposure of mussels Mytilus edulis to crude oil.

We conducted tests to determine whether byssal thread extrusion by Mytilus edulis is a sensitive indicator of exposure to pollutants. Blue mussels (16,3-25.1 mm) were glued to plates (four per plate), and the plates were stacked in tanks (six plates per tank). Mussels in the tanks were exposed for 28 days or 48 days to seven concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil. Concentrations of aromatic hydrocarbons in the WSF were 0-2.1 ppm. The 28-day LC50 was 1.4 ± 0.3 ppm (mean ± 95% confidence interval). Byssal thread production for each 24-h or 48-h period was inversely proportional to concentration of aromatic hydrocarbons. Byssal thread extrusion rates were reduced at 15% of the 7-day LC50. Mussels exposed to higher concentrations of aromatic hydrocarbons produced only one byssal thread in 24 h and two byssal threads in 48 h. The controls produced 16-21 byssal threads after 24 h or48 h.

After 28 days, mussels exposed to higher concentrations of the WSF were returned to clean seawater. After 24 h or 48 h in clean seawater, the number of byssal threads was near or greater than number of byssal threads produced by controls even for mussels exposed to concentrations as high as 90% of the 28-day LC50.

Byssal thread production is a very sensitive indicator of exposure to crude oil, but mussels returned to clean water for 1 day after 28-day exposures to the oil WSF produce the same number of byssal threads as controls.

Brodersen, C. C.

In preparation. Rapid narcosis and delayed mortality in larvae of king crab and kelp shrimp exposed to the water-soluble fraction of crude oil.

Larvae of king crab (Paralithodes camtschatica) and kelp shrimp (Eualus suckleyi) were exposed to the water-soluble fraction of crude oil to determine the length of exposure required to kill them. Static bioassay exposure times were from 20 min to 96 h. For larvae of both species, narcosis severe enough to prevent swimming occurred almost immediately, even at low exposure concentrations. Half of the shrimp larvae exposed to 1 ppm of aromatic hydrocarbons for 20 min and half of the crab larvae exposed to 0.5 ppm for 20 min ceased swimming by the end of the exposure. Larvae in the ocean that stop swimming and sink to the bottom are likely to be eaten, injured, or buried; therefore, exposures causing narcosis are likely to be as fatal as longer or stronger exposures that cause death directly. Half of the larvae of either species exposed to 8 ppm of aromatic hydrocarbons for 6 h died, as did half of the larvae exposed to 1-3 ppm for 24 h. In contrast to narcosis, however, death occurred very slowly for exposed larvae. Most larvae did not die until after the exposure had ended.

Brodersen, C. C., and M. G. CarIs.

In preparation. Sensitivity of egg, larval, and adult coonstripe shrimp (Pandalus hypsinotus) to long-term exposure to the water-soluble fraction of Cook Inlet crude oil.

Several life stages of coonstripe shrimp (Pandalus hypsinotus) were exposed to the water-soluble fraction (WSF) of Cook Inlet crude oil in continuous-flow exposures. The LC50's of the WSF's were measured in parts per million aromatic hydrocarbons. Gravid females had a 96-h LC50 of 1.4 ppm (range, 1.2-1.5 ppm) and a 24-day LC50 of 0.54 ppm (range, 0.40-0.68 ppm). Adult female shrimp died continuously throughout the exposure period; however, none died when returned to clean water.

Late-stage eggs were exposed during the month before hatching while they were still being carried by the females. The eggs of all surviving females hatched into swimming larvae, and the larvae were physiologically more resistant than females to the WSF. However, the eggs must be considered to have the same LC50's as the females because eggs cannot survive without the females.

Larvae at different stages of development were exposed to a variety of concentrations and exposure periods. Exposures ranged from a minimum of 24 h (timed to fall between molts) to a 30-day exposure that covered three molts. Unlike adults, larvae seemed unaffected by the length of exposure. All tests with larvae gave similar results: 1.3 ppm of aromatic hydrocarbons killed them; 0.6 ppm did not.

Brodersen, C. G., and S. D. Rice.

In preparation. Comparison of static and continuous-flow exposure methods for exposing marine animals to toluene, naphthalene, and the water-soluble fraction of crude oil.

Several species were exposed for 96 h to toxicants in either static or continuous-flow tests. Dead animals were counted after each exposure and after the animals were held in clean seawater. Results from the two types of exposures were compared.

Different species responded differently to the toxicants. Pink salmon fry and kelp shrimp were quickly affected and soon died. Purple shore crabs and Hall's colus (Colus halli) were affected quickly but did not die immediately after being damaged; however, longer exposure periods increased the toxicity of the compounds tested. Blue mussels and tarspot cucumbers (Cucumaria vegae) resisted exposure for the first day and then responded very slowly. Once the mussels and cucumbers were affected, they were even slower to die.

Concentrations of toxicants decreased rapidly in static solutions but remained constant in continuous-flow solutions. In assays with individual hydrocarbons, animals that were quickly affected (pink salmon fry and kelp shrimp) had similar results in the two types of tests, whether dead animals were counted at the end of the exposure or after being held in clean seawater. Results of static tests with the WSF and animals resistant to the toxicants were different from results of flow-through tests with the same toxicants and animals. The proportions of crude oil WSF delivered by the static method and by the continuous-flow method were too different to give similar results even in assays of pink salmon fry and kelp shrimp,

Brodersen, C. C., S. D. Rice, J. W. Short, T. A. Mecklenburg, and J. F. Karinen.

1977. Sensitivity of larval and adult Alaskan shrimp and crabs to acute exposures of the water-soluble fraction of Cook Inlet crude oil. In Proceedings 1977 oil spill conference (prevention, behavior, control, cleanup), p. 575-578. American Petroleum Institute, Washington D.C.

The sensitivity of adult and larval Alaskan shrimp and crabs to the water-soluble fraction (WSF) of Cook Inlet crude oil was measured by tests using 96-h static tests at the water temperatures that these animals normally encounter. Larval crustaceans were found to die more slowly than adults, making it necessary to measure sensitivity in terms of concentrations causing moribundity (death imminent) instead of in terms of concentrations causing death during exposure. The cessation of all motion and reaction indicated moribundity in adults, and the cessation of swimming indicated moribundity in larvae exposed for 96 h. The 96-h LC50'S for moribundity for Stage I larvae ranged from 0.95 to 1.8 ppm, depending on species, whereas 96-h LC50'S for adults ranged from 1.9 to 4.2 ppm oil. Sensitivities for Stages I-VI larvae of coonstripe shrimp ranged between 0.24 and 1.9 ppm.

Larvae were more sensitive to oil than adults. The sensitivity of larvae depended on species and developmental stage. Larvae are probably more vulnerable than adults to oil exposure because of greater sensitivity to oil and greater susceptibility to predation. Coldwater species may be particularly vulnerable because of increased time spent as developing larvae.

CarIs, M. G., and S. Kern.

In press. Sensitivity of arctic marine amphipods and fish to petroleum hydrocarbons. Can. Fish. Aquat. Sci. Tech. Rep.

We tested the sensitivities of several arctic marine species to petroleum hydrocarbons and compared them to the sensitivities of temperate species previously tested using the same flow-through procedures and toxicants. We restricted our comparisons between arctic and temperate species to experimental data collected within our laboratory in order to avoid the problems caused by variations in techniques and toxicants which plague oil toxicity research.

We examined two alternative hypotheses: 1) marine arctic animals, adapted to a wide range of environmental parameters, are unusually resistant to unaccustomed stresses such as petroleum hydrocarbons; or 2) marine arctic animals are unusually sensitive to hydrocarbon stress because they are already stressed to their limits by the environment in which they live.

We determined the sensitivities to WSF's of Cook Inlet crude oil 'or naphthalene for six circumpolar benthic species: The amphipods Anonyx nugax, Boeckosimus nanseni, and Gammaracanthus loricatus; a mysid, Mysis relicts; Arctic cod, Boreogadus saida; and a sculpin, Oncottus hexacornis. Exposures were flow-through and lasted up to 40 days. Median lethal concentrations (LC50's) of the WSF ranged from 1.6 to 3.8 ppm total aromatics. Naphthalene assays were conducted at several different temperatures (ranging from 1.5 to 9.6°C) to study temperature effects on sensitivity to hydrocarbons. Upper lethal temperatures for the amphipods and mysid were surprisingly high: 17-24°C, suggesting the assay temperatures in themselves were not particularly stressful. Naphthalene LC50's ranged from 1.35 to 3.35 ppm. General relationships between exposure temperatures and LC50'S were not found.

We conclude that arctic species are about equal in sensitivity to temperate species. However, their habitat is more vulnerable to the effects of petroleum hydrocarbon pollution than temperate habitats because low temperatures lead to slower losses of hydrocarbons from volatilization and biodegradation, and oil entrapment under sea ice can result in very lengthy exposures. Once physical or chemical perturbations have caused damage to habitat and decreases in animal populations, recovery and re-establishment of communities may be slow because of low productivity, low species diversities, and slow growth rates (Dunbar 1968; Grainger 1975; Wacasey 1975).

Carls, M. G., and S. D. Rice.

1984. Toxic contributions of specific drilling mud components to larval shrimp and crabs. Mar. Environ. Res. 12:45-62

We investigated the toxicities of six drilling muds, toxicities of mud fractions (supernatants and suspensions), and the toxicities of common mud components--barite and bentonite (particulate) and ferrochrome lignosulfonate (soluble)--to the stage I larvae of six species of shrimp and crab. The drilling muds we tested were not very toxic to these larvae: LC50's for supernatants ranged from 0.6 to 82% (vol/vol). Shrimp larvae were slightly more sensitive than crab larvae.

Drilling muds were not rapidly toxic, in contrast to toxicants such as the water-soluble fractions of oil. Supernatants, prepared by centrifuging whole muds, were mildly toxic. Suspensions were more toxic than supernatants, and toxicity was greatest when particulate remained suspended: for example, used Cook Inlet mud suspensions were about seven times more toxic than supernatants. The toxicity of used Cook Inlet mud was therefore primarily due to suspended solids (88%) rather than chemical toxicity: ferrochrome lignosulfonate was relatively toxic alone but accounted for only about 6% of the toxicity of used Cook Inlet mud suspensions. Contributions of particulate to mud toxicities varied considerably. Barite and bentonite were not very toxic when tested alone. The toxicity of one mud was caused by its high alkalinity.

CarIs, M. G., and S. D. Rice.

In preparation. Comparative stage sensitivities of walleye pollock, Theragra chalcogramma, to external hydrocarbon stressors.

Fish larvae are often more sensitive to petroleum hydrocarbons than eggs, yet sensitivity is usually very early in development when damage to a few precursor cells results in extensive damage as the embryo develops. In our studies with walleye pollock (Theragra chalcogramma) embryos, concentrations of petroleum hydrocarbons in tissues were lower before hatching than after hatching. With concentrations adjusted to tissue levels, the developing embryos were more sensitive to hydrocarbons before hatching if concentrations of hydrocarbons were measured as tissue concentrations.

Eggs exposed to hydrocarbons had slower development, greater mortality, more variable hatching time, and the resulting larvae were shorter and had more morphological abnormalities than controls. Larvae exposed to the hydrocarbons did not have morphological abnormalities; however, the concentration that killed half of the larvae was the same as the concentration that produced abnormalities when embryos were exposed. Surprisingly, exposures that began at fertilization and lasted for the 2-h water-hardening period had no discernible effects.

The deformed larvae would probably have lower survival in nature because they would have difficulty feeding and avoiding predators.

Cheatham, D. L., and S. D. Rice.

In preparation. The relative importance of evaporation and biodegradation temperature on the loss of some mononuclear and dinuclear aromatic hydrocarbons from seawater.

The seawater-soluble fraction (WSF) of Cook Inlet crude oil was held at 5°, 8°, and 12°C for 96 h. During the 96 h, samples of the WSF were analyzed by gas chromatography to determine the effect of temperature on evaporation and biodegradation of mononuclear and dinuclear aromatic hydrocarbons. We separated losses from evaporation and biodegradation by aerating some solutions and by killing microorganisms in others.

At lower temperatures, mononuclear aromatic hydrocarbons were primarily lost through evaporation rather than biodegradation. Biodegradation, however, was important in the loss of dinuclear aromatic hydrocarbons, particularly naphthalene. Although aromatic hydrocarbons evaporate or are biodegraded at low temperatures, oil and seawater mixtures could be more toxic longer at lower temperatures because aromatic hydrocarbons would persist longer.

Evans, D. R., and S. D. Rice,

1974. Effects of oil on marine ecosystems: a review for administrators and policy makers. U.S. Natl. Mar. Fish. Serv., Fish. Bull. 72:625-638.

A broad selection of recent literature on the effects of oil on marine ecosystems is reviewed. The focus is on studies on crude oil, and the results are discussed with the purpose of providing a summary of findings that will be a useful reference for administrators and policy makers involved in decisions concerning petroleum developments and related activities. The characteristics of crude oil and factors modifying its impacts on the marine environment are discussed. Most research on the toxicity of oil has dealt with acute effects, and data on long-term impacts at the community level are inconclusive. It is concluded that chronic low-level pollution is potentially more damaging to ecosystems than isolated catastrophic spills. Decision makers are forced to rely on interpretative judgments rather than conclusive data.

Faris, T. L., S. D. Rice, and M. M. Babcock.

In preparation. Reduced byssal thread extrusion by the mussel Mytilus edulis during short-term exposure to toluene, naphthalene, and the water-soluble fraction of crude oil.

To determine whether byssal thread production by blue mussels is an indicator of sublethal response to hydrocarbons, we exposed these animals to five concentrations each of naphthalene, toluene, and the water-soluble fraction of Cook Inlet crude oil and No. 2 diesel fuel in a series of 96-h exposures. In previous studies, byssal thread production was enhanced at concentrations lower than those that were inhibitory. At 24, 48, 72, and 96 h, cumulative number of byssal threads produced was significantly depressed in all but the lowest doses of toxicant. After 24 h of exposure, however, the number of new byssal threads extruded during a 24-h period was the similar to the number extruded by controls.. By 72 h, toxicant concentrations decreased because of evaporation, and the rate byssal threads were extruded increased and sometimes exceeded the control rate.

Gharrett, J. A., and S. D. Rice.

In preparation-a. Temperature modification of uptake and deputation of two petroleum hydrocarbons in four marine species.

Acute toxicity, accumulation, and deputation of naphthalene and toluene at 4° and 12°C were determined for pink salmon fry, the purple shore crab, a subtidal snail Colus jordani, and kelp shrimp. All tests were continuous flow, and tissues or whole animals were sampled as appropriate.

The effect of temperature on sensitivity of animals varied with species and toxicant. Snails and kelp shrimp were more sensitive to both toxicants at 12° than at 4°C. Purple shore crabs, however, were more sensitive to naphthalene at 4°C than at 12°C. Sensitivity to toluene was similar at both temperatures. Pink salmon fry were more sensitive to both toxicants at 4°C than at 12°C.

Temperature did not affect the amount of toluene accumulated in any tissues, except crab gills, or the amount depurated by any species. Three to four times more toluene was accumulated in crab gills at 12° than at 4°C.

In some cases, temperature affected the uptake of naphthalene. Tissues of snails and salmon fry initially took up naphthalene more rapidly at 12°C, but the total concentration accumulated was higher at 4° than at 12°C. The reverse was true for purple shore crabs and kelp shrimp, which accumulated more naphthalene at 12°C than at 4"C. Temperature did not affect deputation of naphthalene by any species or tissue.

Regardless of temperature, naphthalene was always more toxic than toluene and accumulated to higher concentrations. The rates of deputation were also similar for the two toxicants, regardless of temperature.

Gharrett, J. A., and S. D. Rice.

In preparation-b. Intermittent aerial exposure and the toxicity, uptake, and deputation of aromatic hydrocarbons in the purple shore crab Hemigrapsus nudus.

The purple shore crab was used to determine the relationship between intermittent aerial exposure and toxicity, accumulation, and deputation of two aromatic hydrocarbons, toluene and naphthalene. Three tidal regimes were studied in which crabs spent O, 33, or 66% of the time aerially exposed and the remainder of time in different concentrations of toxicant solutions.

Aerial exposure significantly affected sensitivity, bioaccumulation, and deputation of toluene and naphthalene. Because the crab only accumulated toxicants during immersion, sensitivity was greatest for crabs that were immersed longest.

Naphthalene was more toxic than toluene in all exposure regimes. Tissue concentrations of naphthalene increased faster and, at the end of the exposures, were 10 times higher than tissue concentrations of toluene. The LC50 for naphthalene was one tenth that for toluene whether effects were measured by the number of dead crabs, the number of crabs with locomotor abnormalities, or exposure time before death. Deputation was greatest in crabs immersed longest in clean water; therefore, recovery potential was reduced in aerially exposed crabs that already had high concentrations of hydrocarbons in their tissues.

Significantly higher respiration rates of crabs in air (as opposed to rates for the same crabs in water) indicated that reduced accumulation and deputation with aerial exposure was not due to a general metabolic quiescence.

Karinen, J. F.

1977. Assessing oil impacts with laboratory data application, limitations, and needs. In B. Melteff (editor), Oil and aquatic ecosystems, tanker safety and oil pollution liability, Proceedings of the Cordova Fisheries Institute, p. 99-110. University of Alaska, Fairbanks, Sea Grant Rep. 77-8.

This paper outlines some of the problems associated with the utilization of laboratory data to assess the impact of oil in the environment and some of the considerations which we should make in trying to apply the toxicity and sublethal effects data. In applying these results to the environment to determine what the actual effects of oil exposure will be, there are both biological as well as chemical considerations which we must take into account. This paper deals more with the chemical aspects rather than the biological aspects (although I fully realize the great importance of the biological relationships) and is limited to a consideration of the behavior of oil in water and some chemical factors influencing the application of laboratory effects data toward assessing oil impacts.

Karinen, J. F.

1980. Petroleum in the deep sea environment: potential for damage to biota. Environ. Int. 3:135-144.

Information on the fate, persistence, and biological impact of petroleum hydrocarbons in shallow marine environments, coupled with recent data on hydrocarbons in offshore sediments and the biology of deep-sea organisms, have provided new perspectives on the potential impact of oil on the deep-sea environment. A review of literature on petroleum hydrocarbons in deep-sea sediments, mechanisms for transport of petroleum to the deep-sea floor, interaction of petroleum hydrocarbons and particulate matter, and physiology and metabolism of deep-sea fish and crustaceans has resulted in the following conclusions:

1. Hydrocarbons of apparent anthropogenic origin are accumulating in bottom sediments of coastal margins and in deeper offshore waters at unknown rates.

2. Several mechanisms exist for the rapid transport of petroleum hydrocarbons to the deep-sea floor.

3. Petroleum hydrocarbons are intimately associated with particulate matter in the sea and behave much the same as natural biogenic material and have the potential to modify or interrupt natural processes.

4. The unique physiology of deep-water life forms increases the potential for adverse impact of petroleum hydrocarbons in the deep-sea environment.

5. There is a need to determine trends of temporal and spatial deposition of hydrocarbons in deep-sea sediments and evaluate the biological impact of this introduction of xenobiotic compounds on the largest environment on earth.

Karinen, J. F., and S. D. Rice.

1974. Effects of Prudhoe Bay crude oil on molting Tanner crabs, Chionoecetes bairdi. U.S. Natl. Mar. Fish. Serv., Mar. Fish. Rev. 36(7):31-37.

Pre- and post-molt juvenile male Tanner crabs, Chionoecetes bairdi, from Alaskan waters were exposed to Prudhoe Bay crude oil in static tests in the laboratory. Crabs in both stages were similarly susceptible to crude oil; the estimated 48-h median tolerance limits values were 0.56 ml oil/liter. Molting "success decreased with increasing exposure of crabs to oil, and newly molted crabs autotomized limbs during exposure to oil. Relating the results of our study to the known behavior of crabs and the documented behavior of oil spills in the ocean suggests that oil spilled in Alaskan waters would harm the Tanner crab resources. The impact on all crab resources of chronic low-level oil pollution from the ballast water discharged into Prince William Sound is unknown. This study further illustrates our present state of ignorance concerning the biological effects of oil in the marine environment.

Karinen, J. F., G. Perkins, R. T. Myren, and W. D. MacLeod, Jr.

In preparation-a. Survival and uptake of hydrocarbons by mussels, pink salmon fry, and kelp shrimp caged near the treated-ballast water diffuser, Port Valdez, Alaska.

We determined the survival of blue mussels, pink salmon fry, and juvenile kelp shrimp. that were caged and suspended in the plume of treated ballast water extending from the diffuser of the ballast-treatment facility at Port Valdez, the terminus of the trans-Alaska pipeline. Salmon fry and shrimp were exposed for 8 days; mussels were exposed for up to 30 days.

Hydrocarbon content of the exposed animals was determined to identify the major toxicants in the effluent and to correlate toxicity with the amount of hydrocarbons in the tissues. We also determined the concentration of aromatic hydrocarbons adsorbed on suspended sediments within and outside the plume by collecting sediment samples from several stations in Port Valdez.

Although some of the caged animals died, no deaths were attributable to hydrocarbons. Concentrations of hydrocarbons greater than background levels were found only in blue mussels from cages closest to the diffuser.

Karinen, J. F., L. S. Ramos, and W. D. MacLeod, Jr.

In preparation-b. Hydrocarbon distribution in the intertidal marine environment of Port Valdez and Prince William Sound, Alaska.

Prince William Sound, in the northern part of the Gulf of Alaska, is an important fishing area of about 2,500 square miles. Two months before the first crude oil was shipped from Port Valdez, the terminus of the Trans-Alaska Pipeline, we began monitoring 10 intertidal sites in Prince William Sound and Port Valdez to determine annual fluctuations in petroleum hydrocarbon content of blue mussels, yellowfin sole, and sediments.

The hydrocarbon content varied depending on the site, the date of collection, and the type of sample. Sediments and biota usually had very low concentrations of petroleum hydrocarbons, except for one harbor site in Port Valdez, and typified the low hydrocarbon concentrations expected in a pristine environment.

Kern, S., and S. Rice.

1981. Sensitivity to, and accumulation and deputation of, aromatic petroleum components by early life stages of coho salmon (Oncorhynchus kisutch). Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:87-92.

Coho salmon eggs, alevins, and fry were exposed to toluene, naphthalene, and l-rnethy lnaphthalene (aromatic hydrocarbons found in crude oil) in a series of short-term toxicity and hydrocarbon-uptake studies to determine whether acute toxicity is related to uptake-depuration patterns. Uptake studies used radio-labeled compounds and radiometric analyses. The time to reach maximum tissue concentrations of these hydrocarbons was determined from long-term exposures.

Sensitivity to the aromatic hydrocarbons increased from egg to fry with the greatest increase in sensitivity between the egg and early alevin. The rates of uptake and deputation of the aromatic hydrocarbons also increased during the development from egg to fry. Eggs had the slowest rates of uptake and deputation.

Eggs required 10 days to accumulate stable tissue concentrations of toluene and naphthalene; alevins required 36 h (both toxicants); fry required 3 h (toluene) and 10 h (naphthalene). The rate of uptake and toxicity was higher with increased ring size and increased substitution (2-methylnaphthalene > naphthalene > toluene).

Eggs were more tolerant than alevins and fry to short-term exposures of aromatic hydrocarbons probably because the chorion prevented rapid uptake. The amount of yolk also influenced sensitivity because aromatic hydrocarbons were selectively partitioned into the yolk thus reducing availability of the hydrocarbons to the embryo and resulting in lower toxicity.

Although eggs take up hydrocarbons at a slow rate, they may accumulate lethal levels of hydrocarbons during long-term exposures and, therefore, would be more sensitive to hydrocarbons than indicated by short-term experiments.

Kern, S., S. A. Lindsay, and S. D. Rice.

In preparation. Accumulation and deputation of the water-soluble fraction of Cook Inlet crude oil in eggs and muscle tissue of adult kelp shrimp.

Gravid kelp shrimp were continuously exposed to the water-soluble fraction of Cook Inlet for 10 days. Muscle tissue and eggs were sampled periodically and analyzed for aromatic hydrocarbons by gas chromatography. Muscles and eggs rapidly accumulated hydrocarbons, and maximum concentrations were reached after 3-24 h in muscle and after 48 h in eggs. Concentrations of aromatic hydrocarbons were 10 times greater in the eggs than in muscle. Of the aromatic hydrocarbons found in the eggs and muscle, substituted benzenes had the highest concentration followed by xylenes, toluene, and 2-methylnaphthalene. Phenanthrene was also found but accumulated steadily only after 4 days.

The rate hydrocarbons were depurated varied. Toluene persisted in eggs and muscle, and small amounts were depurated only after 4 days. Other monoaromatic hydrocarbons and naphthalene were eliminated rapidly, and less than 30% remained after 4 days. Methylnaphthalene and methylated benzenes had intermediate rates of deputation.

Exposure to oil probably affects fertilization, development, and hatching of shrimp eggs due to rapid accumulation and slow deputation of certain aromatic hydrocarbons.

Kern, S., D. A. Moles, and S. D. Rice.

1979. Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene, and Cook Inlet crude oil. Bull. Environ. Contain. Toxicol. 21:521-525.

A series of static 96-h tests at different temperatures was run with toluene, naphthalene, and the water-soluble fraction of Cook Inlet crude oil using pink salmon and Eualus shrimp. The 96-h median tolerance limit (TLm) of pink salmon exposed to toluene was significantly lower at 4° than at 12°C. The 96-h TLm's for shrimp exposed to toluene and naphthalene were significantly higher at 4°C than at 12°C. Other tests were not significant. We concluded that temperature affects the sensitivity of oil components to pink salmon and shrimp in a nonuniform way, and this effect should be tested for each component with each organism.

Kern, S., S. D. Rice, D. L. Cheatham, and D. W. Brown.

In press. Contribution of phenol, and p-cresol to the toxicity of crude oil to (Oncorhynchus gorbuscha) fry and kelp shrimp (Eualus suckleyi). In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Symposium on pollution and physiology of marine organisms, Mystic, Corm., Nov. 1983. University of South Carolina Press.

Although aromatic hydrocarbons are presumed to be the major contributors to the toxicity of oil-water solutions, the quantitative contribution of nonaromatic and aromatic compounds is undocumented. The objective of this research is to measure the contribution of the highly water-soluble phenolic compounds to toxicity of water-soluble fractions (WSF) of oil.

Phenol and p-cresol do not contribute greatly to the toxicity of the WSF probably because concentrations of phenols in the WSF are low, their toxicity is relatively low, and the relatively low accumulation and rapid deputation of phenol and cresol compared with the important oil aromatic hydrocarbons, toluene and naphthalene.

Phenol and phenolic compounds were found in the WSF of Cook Inlet crude oil at concentrations of 0.013-0.092 ppm. The acute toxicity of phenol and cresol to pink salmon was 3.73 and 3.36 ppm, respectively, and with shrimp, 10.31 and 7.36 ppm, respectively. Salmon and shrimp accumulated 11-22 times the initial exposure concentration of phenol and cresol in 24 h. Both compounds were eliminated rapidly with residues being undetectable after 7 days or less.

Lauren, D. J., and S. D. Rice.

In preparation. Uptake, deputation, metabolism, and excretion of naphthalene by the purple shore crab, Hemigrapsus nudus.

Adult male shore crabs were exposed statically in seawater to ^{14}C-labeled naphthalene for 12 h, followed by up to 156 h of deputation. Hemolymph, heart, cardiac and pyloric stomachs, muscle, thoracic ganglion, digestive gland, and gills were removed at intervals and analyzed for the accumulation of ^{14}C-labeled naphthalene and its metabolizes. Biomagnification was rapid, and by 12 h, the digestive gland had 105 times the seawater concentrations of carbon-14, whereas other tissues had less than 15 times the seawater concentration. Deputation was rapid at first but slowed by 12 h after exposure. After 156 h of deputation, the parent naphthalene that remained was located primarily in the digestive gland and muscle; the highest percentages of naphthalene metabolizes were found in the gills, muscle, and hemolymph. Because no significant difference in deputation rate was found between control crabs injected with ^{14}C-labeled naphthalene and those with their nephropores and anus blocked, it was concluded that the gills are the major route of naphthalene elimination. Thin-layer chromatography of extracts of depurated ^{14}C-labeled naphthalene indicated that <10% of the total naphthalene depurated was metabolizes. In vitro mixed function oxygenase activity (MFO) was assayed on centrifuged (15,000 g) tissue homogenates using dipheyloxazole as the terminal electron acceptor. The specific activity of the gills was 2-4 times that of the antennal glands, and no activity was detected in either muscle, digestive glands, or cardiac and pyloric stomachs. Furthermore, MFO activities were very low compared to fish or mammals. Thus, it was concluded that metabolism of naphthalene plays a minor role in the reduction of toxic body burden, the major role being played by simple diffusion of unmetabolized naphthalene down a concentration gradient. This occurs across the tissue with the largest body-to-water surface area, the gill.

Mecklenburg, T. A., J. F. Karinen, and S. D. Rice.

In preparation. Decrease in heart rates of the Alaskan king crab (Paralithodes camtschatica) during exposure to Cook Inlet crude oil, benzene, and naphthalene.

Heart rates of king crab were depressed during exposure to seawater-soluble fractions (WSF's) of Cook Inlet crude oil, benzene, and naphthalene. As concentrations of crude oil or aromatic concentrations in the seawater declined, the heart rate increased; thus, heart rate of king crabs can be a sensitive indicator of hydrocarbon stress. In one of the experiments with crude oil, changes in respiration closely paralleled changes in heart rate. Benzene produced quicker, more severe, and longer-lasting depression of the heart rate than naphthalene or crude oil. The long-lasting sublethal effect of benzene was evident even though the benzene degraded more rapidly in the water than either crude oil or naphthalene.

Mecklenburg, T. Il., S. D. Rice, and J. F. Karinen.

1977. Molting and survival of king crab (Paralithodes camtschatica) and coonstripe shrimp (Pandalus hypsinotus) larvae exposed to Cook Inlet crude oil water-soluble fraction. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 221-228. Pergamon Press, New York.

Larvae of coonstripe shrimp and king crab were exposed to solutions of the water-soluble fraction (WSF) of Cook Inlet crude oil in a series of tests on intermolt Stages I and II and the molt period from Stage I to Stage II. Molting larvae were more sensitive than intermolt larvae to the WSF, and molting coonstripe shrimp were more sensitive than molting king crab larvae. When molting larvae were exposed to high concentrations of the WSF (1. 15-1.87 ppm total hydrocarbons) for as little as 6 h, molting success was reduced by 10-30%, and some deaths occurred. When larvae were exposed to these high concentrations for 24 h or longer, molting declined 90-100%, and the larvae usually died. The lowest concentrations tested (0.15-0.55 ppm total hydrocarbons) did not inhibit molting at any length of exposure, but many larvae died after molting. Median lethal concentrations (LC50's) based on 144 h of observation for molting coonstripe shrimp and 120 h for molting king crab were much lower than the 96-h LC50's, showing that the standard 96-h LC50 is not always sufficient for determining acute oil toxicity. Although our LC50's for intermolt larvae are higher than levels of petroleum hydrocarbons reported for chronic and spill situations, some of our LC50'S for molting larvae exposed 24 h and longer are similar to or below these environmental levels. Comparisons of sensitivity to oil between different crustacean species or life stages should be based on animals tested in the same stage of the molt cycle, such as intermolt.

Moles, A.

1980. Sensitivity of parasitized coho salmon fry to crude oil, toluene, and naphthalene. Trans. Am. Fish. Sot. 109:293-297.

The effect of parasitism by glochidia of Anodonta oregonensis (a freshwater mussel) on the sensitivity of coho salmon fry, Oncorhynchus kisutch, to oil was determined by exposing fry with different levels of parasitism to several concentrations of either the water-soluble fraction of Prudhoe Bay crude oil or the aromatic hydrocarbons toluene and naphthalene. Fry infested with 20-35 glochidia were significantly ($P < 0.05$) more sensitive to each of the toxicants than uninfested fish. Sensitivity increased linearly with increased parasite numbers. Interpretation and application of results of toxicity tests should take into account the kinds and intensities of parasitism found both in the test animals and in the wild populations of fish.

Moles, A. and S. D. Rice.

1983. Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater. Trans. Am. Fish. Sot. 112:205-211.

Juvenile pink salmon, Oncorhynchus gorbuscha, were exposed for 40 days to stable, sublethal concentrations of naphthalene (<0.80 mg/1) and the water-soluble fraction (WSF) of Cook Inlet crude oil (<0.87 mg/1 total aromatic hydrocarbons). All fish were fed equal daily rations of Oregon Moist Pellets Formula II. Concentrations (percentage of the 96-h LC50, median lethal concentration) of 10% naphthalene and 14% WSF of crude oil did not affect weight or length of exposed fish; however, at higher concentrations, growth per day, determined from dry weight, decreased with increased toxicant concentration. Fish exposed for 40 days to concentrations of toxicants as low as 33% of the 96-h LC50 were significantly smaller in dry weight, wet weight, and length than control fish ($P < 0.05$). Juveniles exposed to the WSF of crude oil had slower growth rates than those exposed to the same concentration (percentage of the LC50) of naphthalene. Fish exposed to either naphthalene or the WSF of crude oil had decreased caloric content; however, fat content of the fish was not affected. Chronic marine oil pollution at a concentration as low as 0.40 mg/1 total aromatic hydrocarbons could be detrimental to juvenile pink salmon growth.

Moles, D. A., and S. D. Rice.

In preparation. The sensitivity of early life stages of coho salmon to long-term exposures to crude oil, toluene, and naphthalene.

Length of exposure affected the concentration of toluene, naphthalene, or crude oil that killed coho salmon eggs, alevins, and fry. The differences are most. pronounced in early alevins and least pronounced in fry, and test animals continued to die after they were placed in clean water. The number that died after the exposures was related to length of previous exposure and amount of yolk present during the exposure.

Moles, D. A., S. D. Rice, and S. A. Andrews.

In press. Continuous-flow devices for exposing marine organisms to the water-soluble fraction of crude oil and its components. Can. Fish. Aquat. Sci. Tech. Rep.

The devices produce stable concentrations of aromatic hydrocarbons that can be used in continuous-flow toxicity tests. The crude-oil mixing device produces a stable (<5% deviation) water-soluble fraction of 2.5 mg/l total aromatic hydrocarbons for 30-40 days. The device uses a gentle flow of water to dissolve aromatic components in a layer of crude oil floating on a 2-m column of seawater. Because the water does not pick up oil droplets as it passes through the column, a water-soluble fraction is produced rather than a dispersion. The other device, a syringe pump, introduces compounds directly into a water stream and produces a stable (<1% deviation for toluene) solution of monoaromatic hydrocarbons of any desired concentration or mixture up to the maximum volubility of the compounds. Both devices give reproducible results, are inexpensive, easily maintained, safe, and adaptable to many toxicants.

Moles, D. A., M. M. Babcock, and S. D. Rice.'

In preparation. Effects of simulated intertidal crude oil exposures on the survival, development, and uptake in pink salmon (Oncorhynchus gorbuscha) alevins.

Pink salmon (Oncorhynchus gorbuscha) often spawn intertidally in the lower reaches of freshwater streams where developing eggs and embryos of these fish are vulnerable to the possible interaction of varying salinity and oil pollution. We exposed 5-day- and 60-day-old alevins to concentrations of Cook Inlet crude oil in fresh water and in a simulated tidal cycle. Only the higher concentrations (1.6-2.4 mg/l total aromatic hydrocarbons) in the intertidal regime reduced survival. Size was reduced by both changing salinity and exposure to oil, and 60-day-old alevins were more severely affected than the 5-day-old alevins. Even a few hours of daily exposure to oil reduced size. The 60-day-old alevins in the simulated tidal cycle accumulated the highest concentrations of petroleum hydrocarbons.

Alevins exposed intermittently to oil, regardless of salinity, sequestered few hydrocarbons in their tissues. In conclusion, pink salmon alevins in intertidal substrates would be more adversely affected by exposure to oil than their freshwater counterparts.

Moles, D. A., S. Bates, S. D. Rice, and S. Kern.

1981. Reduced growth of coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water. Trans. Am. Fish. Sot. 110:430-436.

Coho salmon, Oncorhynchus gorbuscha, fry were exposed 40 days to stable, sublethal concentrations of toluene (0.4, 0.8, 1.6, 3.2, 5.8 ml/1) and naphthalene (0.2, 0.4, 0.7, 1.4 mg/1) in fresh water. All fry were fed equal daily rations of Oregon Moist Pellet Formula II. Dry weights, wet weights, and lengths of fry exposed to the two highest concentrations of each toxicant for 40 days were significantly less than controls ($P < 0.01$). Growth per day, determined from weights and lengths, decreased linearly with increased concentrations. Fry exposed to naphthalene had a slower growth rate than fry exposed to equivalent concentrations (percentage of the 96-h median lethal concentration of LC50) of toluene. Concentrations 18% of the LC50 of naphthalene and 26% of the LC50 of toluene had no effect on dry weight, wet weight, or length of exposed fry.

Moles, D. A., S. D. Rice, and S. Kern.

1979. Sensitivity of Alaskan freshwater and anadromous fishes to Prudhoe Bay crude oil and benzene. Trans. Am. Fish. Sot. 108:408-414.

The sensitivity of various species and life stages of Alaskan freshwater and anadromous fishes to benzene and the water-soluble fraction of Prudhoe Bay crude oil was determined with 96-h toxicity tests. Freshwater juveniles of the six salmonid species tested had similar sensitivities. Median tolerance limits (TLm's) of these salmonids for crude oil ranged from 2.7 to 4.4 mg/l; TLm's of benzene ranged from 11.7 to 14.7 ml/1. Threespine sticklebacks and, to a lesser extent, slimy sculpins were more tolerant than salmonids and had larger TLm's: threespine sticklebacks had a crude-oil TLm of 10.4 mg/l and a benzene TLm of 24.8 ml/l; slimy sculpins had a crude-oil TLm of 6.44 mg/1 and a benzene TLm of 15.4 ml/l. Eggs of pink salmon and coho salmon were quite tolerant to crude oil (TLm = >12 mg/l) and benzene (TLm = 339-542 ml/1). Emergent fry were the most sensitive freshwater stage (crude-oil TLm = 8.0 mg/l; benzene TLm = 12.3-17.1 ml/1). Outmigrant salmonids tested in seawater were twice as sensitive as outmigrant salmonids tested in fresh water apparently because of the additional stress of entering seawater and the physiological changes associated with this transition. Freshwater TLm's were 2.3-8.0 mg/l for crude oil and 10.8-17.1 ml/1 for benzene. Corresponding seawater sensitivities were 1.1-3.6 mg/l for crude oil and 5.5-8.5 ml/1 for benzene.

Myren, R. T., and J. J. Pella.

1977. Natural variability in distribution of an intertidal population of Ma coma balthica subject to potential oil pollution at Port Valdez, Alaska. Mar. Biol. (Berl.) 41:371-382.

Natural variability in the abundance of an intertidal population of the lamellibranch Macoma balthica (Linnaeus 1758) was measured during 1971 and 1972 in a study area near the proposed oil storage and tankship loading facility at the southern terminus of the Trans-Alaska Pipeline in Port Valdez, Alaska. Macoma balthica were divided for analysis into a large- and a small-size category. Small temporal changes in population densities throughout the entire study area were detected for both size categories over several of the seven sampling times of the 2-yr period. Large and persistent differences in density were found among elevation contour intervals for either size category; however, variations in the density profiles on elevation occurred among sampling times. Large M. balthica became more equitably distributed and the small category less equitably distributed among elevation contours over the 2-yr period. Densities of both size categories were more stable at the higher elevations of the study site. Large M. balthica were more homogeneously distributed along a given elevation contour interval than the small category. Mobility and time available to redistribute at a horizontal location would explain the more homogeneous distribution of large M. balthica if competition for food resources exists.

Myren, R. T., and G. Perkins.

In preparation. Decline in Macoma balthica (L.) abundance at Port Valdez, Alaska, 1971-80.

Annual and seasonal changes in abundance of Macoma balthica clams on an intertidal mudflat at Port Valdez, Alaska, were measured over a 10-yr period, 1971-80. Variability in densities of large (>6.4 mm) and small (3.2-6.4 mm) M. balthica changed during highway and oil-terminal construction, and operation of the ballast-water treatment plant and trans-Alaska oil pipeline compared to a 1971-73 baseline observation period. Densities of large M. balthica decreased after the ballast-treatment plant at Port Valdez began operating in 1977, and the lowest densities were recorded in 1980. Effluent from the ballast-water treatment plant is the main source of petroleum hydrocarbons in Port Valdez. A causal relationship, however, was not demonstrated between decreased density of M. balthica and operation of the oil terminal and ballast-water treatment plant. A model was developed to explain variability of M. balthica density during the 10-yr period.

O'Clair, C. E., and J. F. Karinen.

In preparation. Rates of colonization in sublittoral communities near the oil tanker ballast facility at Valdez, Alaska.

Field experiments were conducted for 14 months to determine whether the effluent. from the ballast-water treatment plant at Port Valdez, Alaska, affected the rate marine invertebrates form colonies in Port Valdez. Settling plates of compressed asbestos and pans containing defaunated sediment were bolted to an aluminum frame, which was placed at depths of about 90 m and 150 m. The sediments were protected from disturbance when the frames were lowered to depth or retrieved. Two frames were placed near the effluent diffuser of the ballast treatment plant; two other frames were placed in areas not affected by the effluent.

Rates of colonization on the settling plates were markedly different with depth: shallow stations had higher rates of colonization. Serpulid polychaetes were the most abundant early (at 3 months) colonists on the settling plates. Ectoprocts began appearing at 6 months and predominated at the end of the experiment. Effluent from the ballast treatment plant evidently did not affect the rate of colonization.

Sediments were colonized more slowly than plates. Paraonid and syllid polychaetes began colonizing sediments after the plates were immersed for 3 months. Gnathiid isopods and harpacticoid copepods began appearing at 6 months. Preliminary results show no clear trends in rate of colonization on sediments with respect to depth or effluent from the ballast treatment plant.

O'Clair, C. E., and S. D. Rice.

In press. Inhibition of a predator-prey interaction by crude oil: survival, feeding, growth, and condition of Evasterias troschelii preying on Mytilus edulis (L.). Mar. Biol. (Berl.).

Predation by the starfish Evasterias troschelii (Stimpson, 1862) on the mussel Mytilus edulis (L.) can be a strong biological interaction (sensu Paine, 1980) in the inner marine waters of Alaska. To test the effect of petroleum hydrocarbons on this interaction, we exposed E. troschelii with M. edulis to six concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil in a flow-through bioassay system for 28 days. The starfish were more sensitive than the mussels to the WSF; the LC50 for E. troschelii was 0.82 ppm at day 19. Although no mussels were exposed to WSF for more than 12 days, none died.

Daily feeding rates of Evasterias troschelii decreased with increasing concentration of the WSF, whether the rates were measured as the number of mussels·starfish^{-1}·day^{-1} or dry tissue weight of mussels·starfish^{-1}·day^{-1} (mg/sfd). Feeding rates were significantly reduced at all concentrations ≥0.2 ppm; starfish in the two highest concentrations did not feed. At concentrations of 0.20, 0.28, and 0.72 ppm WSF, starfish fed at rates (mg/sfd) that were, respectively, 53, 37, and 5% of the control rates.

Evasterias troschelii exposed to concentrations of WSF ≥0.20 ppm grew slower than control starfish and those exposed to 0.12 ppm combined. The condition of the pyloric caeca and gonads of the starfish expressed as fresh weight or hepatic and gonadal indices was not significantly affected by exposure to WSF. Chronic low level oil pollution can thus inhibit a strong biological interaction and favor Mytilus edulis, a tolerant prey that is a superior competitor for space in the intertidal region.

O'Clair, C.E., and S. D. Rice.

In preparation. Survival, growth, and movement in plantigrades of *Mytilus edulis* exposed to the water-soluble fraction of Cook Inlet crude oil.

Blue mussel plantigrades collected in nature on nylon ropes were brought to the laboratory and exposed to six concentrations of the water-soluble fraction (WSF) of Cook Inlet crude oil. The tests were made in a flow-through system and lasted for 40 days. Control animals were held in fresh, running seawater. Mortality was estimated from dead *M. edulis* that had unbroken shells. Growth was measured as the change in mean shell length of a sample of the plantigrades on each rope, measured at the beginning and end of the experiment.

Plantigrades in the highest concentration of the WSF (1.40 ppm) had the highest mortality (18.5%), and the 40-day LC50 was 1-7 ppm. At concentrations <1.04 ppm, mortality did not consistently decrease with decreasing concentration. Few plantigrades exposed to ≤0.78 ppm of the WSF died. A layer of bacteria that covered the plantigrades exposed to the WSF, especially concentrations ≥0.78 ppm, may have shielded the plantigrades from some of the toxic effects of the WSF. Plantigrades grew the most in 0.78 ppm and 0.38 ppm of WSF, and plantigrades in 1.40 and 1.04 ppm of WSF (61% of the 40-day LC50) decreased in shell length by the end of the experiment.

Rice, S. D.

1973. Toxicity and avoidance tests with Prudhoe Bay oil and pink salmon fry. In Proceedings of 1973 Joint Conference on Prevention and Control of Oil Spills, p. 667-670. American Petroleum Institute, Washington, D.C.

With the potential of oil pollution harming Alaska's marine resources, experiments were conducted at the National Marine Fisheries Service Auke Bay Laboratory to determine the concentrations of Prudhoe Bay crude oil that are acutely toxic to pink salmon fry in fresh water and seawater and also the concentrations of this oil that the fry would avoid. Observed 96-h TLm values were 88 mg/1 of oil in fresh water, 213 mg/l in seawater in June, and 110 mg/l in seawater in August. Among fry held in seawater, older fry were more susceptible to oil toxicity than younger fry, and older fry were also more sensitive in their detection and avoidance of oil; older fry in seawater avoided oil concentrations as low as 1.6 mg of oil/l of water. The avoidance of oil by salmon fry was quite apparent and suggests that there is potential for oil pollution to change their migration behavior.

Rice, S. D.

1977. A review of oil toxicity studies conducted at the Auke Bay Laboratory. In B. Melteff (editor), Oil and aquatic ecosystems, tanker safety and oil pollution liability, Proceedings of the Cordova Fisheries Institute, 1-3 April 1977, p* 111-113. University of Alaska, Fairbanks, Sea Grant Rep. 77-8.

Although each of our publications has specific conclusions, the two most significant general conclusions are:

1. We have generally found crustacean larvae to be the most sensitive life stage, especially when molting.

2. Alaskan species may be more vulnerable to oil than species from warmer waters since lower temperatures cause toxic aromatic hydrocarbons to persist longer. Temperature effects on oil toxicity and animal sensitivity are complex and warrant further study.

Rice, S. D., S. Kern, C. C. Brodersen, S. A. Lindsay, and S. A. Andrews.

1981. Toxicity of ballast-water treatment effluent to marine organisms at Port Valdez, Alaska. In Proceedings, 1981 Oil Spill Conference, Atlanta, Georgia, 2-5 March, p. 55-61. American Petroleum Institute, Washington, D.C.

Approximately 12 million gallons of oily ballast water is taken ashore and treated daily at the Alyeska treatment plant, where tankers take on crude oil at the terminus of the Trans-Alaska Pipeline System near Valdez, Alaska. Most oil is removed, but some light aromatic hydrocarbons (1-16 ppm) remain in the large volume of discharged effluent. Between May and July, the concentration of aromatic hydrocarbons in the treated effluent (measured by gas chromatography) generally declined as the seasonal temperatures increased. We measured the toxicity of the effluent on site at Valdez. For the larvae o-f crustaceans and of fish, the median lethal concentration (LC50) was between 10 and 20% of treated effluent in 96-h static tests. For salmon fry and shrimp in repeated acute flow-through assays, the LC50 was quite consistent, between 20 and 40% of treated effluent. Because the concentration of aromatic hydrocarbons was much lower in the later tests, but the toxicity of the effluent was not lower, toxicants other than aromatic hydrocarbons must contribute significantly to the toxicity of the effluent from the ballast-water treatment plant.

Rice, S. D., D. A. Moles, andJ. W. Short.

1975. The effect of Prudhoe Bay crude oil on survival and growth of eggs, alevins, and fry of pink salmon, Oncorhynchus gorbuscha. In 1975 Conference on prevention and control of oil pollution, Proceedings, p. 503-507. American Petroleum Institute, Washington, D.C.

Standard 96-h tests with "total" oil solutions in fresh water and seawater determined differences in sensitivity of the developing life stages of pink salmon (Oncorhynchus gorbuscha). Eggs were the most resistant and emergent fry (yolk sac absorbed), the most sensitive to acute 4-day exposures. In fresh water, the 96-h median tolerance limit (TLm) of fry was 0.4 ml oil/liter mixed mechanically (12 ppm as measured in subsurface water by infrared spectrophotometry). In seawater, it was 0.04 ml oil/liter mixed mechanically (6 ppm as measured in subsurface water by infrared spectrophotometry).

Three life stages of alevins were exposed to 10-day sublethal exposures of the water-soluble fraction to determine what doses might affect growth. Growth was affected most severely in alevins exposed during later developmental stages. Decreased growth was observed in fry after 10-day exposures at the lowest dose tests--0.075 ml oil/liter mixed by water agitation (0.73 ppm in subsurface water by infrared spectrophotometry--less than 10% of the 96-h TLm limit for that life stage).

In fresh water, susceptibility of early life history stages of pink salmon to oil pollution is great at the time of emergence (completion of yolk absorption). Susceptibility is even greater in seawater after fry migration.

Rice, S. D., A. Moles, T. L. Taylor, and J. F. Karinen.

1979. Sensitivity of 39 Alaskan marine species to Cook Inlet crude oil and No. 2 fuel oil. In Proceedings 1979 Oil Spill Conference (Prevention, Behavior, Control, Cleanup), p. 549-554. American Petroleum Institute, Washington, D.C.

The sensitivities of 39 subarctic Alaskan species of marine fish and invertebrates to water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil were determined. This is the largest group of animals ever tested under similar test conditions with the same petroleum oils and analytical methods. Organisms tested represent several habitats, 6 phyla, and 39 species, including fish (9), arthropods (9), molluscs (13), echinoderms (4), annelids (2), and nemerteans (2). Sensitivities were determined by 96-h static tests. Concentrations of selected aromatic hydrocarbons were determined by gas chromatography; concentrations of paraffins were determined by infrared spectrophotometry.

Although sensitivity generally increased from lower invertebrates to higher invertebrates, and from higher invertebrates to fish, sensitivity was better correlated to habitat. Pelagic fish and shrimp were the most sensitive animals to Cook Inlet crude oil with 96-h median tolerance limits (TLm's) of 1-3 mg/l total aromatic hydrocarbons. Benthic animals, including fish, crabs, and scallops were moderately tolerant (TLm's to Cook Inlet crude oil of 3-8 mg/l total aromatic hydrocarbons). Intertidal animals, including fish, crabs, starfish, and many molluscs, were the most tolerant forms to the water-soluble fraction of petroleum (TLm's greater than 8-12 mg/l of total aromatic hydrocarbons). Most of the intertidal animals were not killed by static oil exposures. Number 2 fuel oil was more toxic to most species than Cook Inlet crude oil.

Sensitive pelagic animals are not necessarily more vulnerable to oil spills than tolerant forms. Oil may damage intertidal environments more easily, and adverse effects may persist longer than in damaged pelagic environments.

Rice, S. D., J. W. Short, C. C,. Brodersen, T. A. Mecklenburg, D. A. Moles, C. J. Misch, D. L. Cheatham, and J. F. Karinen.

1976a. Acute toxicity and uptake-depuration studies with Cook Inlet crude oil, Prudhoe Bay crude oil, No. 2 fuel oil, and several subarctic marine organisms. NWFC Processed Report, 90 p. Auke Bay Laboratory, Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, P.O. Box 210155, Auke Bay, AK 99821.

1. Our studies do not suggest that major differences exist between the responses of the Alaskan marine animals tested and the responses of marine animals from other areas as reported in the literature.

2. The concentration of oil-in-water dispersions and water-soluble fractions of oil in solutions is dependent on a number of factors including oil volume, confinement of the oil, mixing duration, and mixing energy. As a consequence, we do not attempt to transfer the results of our study to a field situation (including Kachemak Bay) or to establish the potential effects of oil contamination on the environment. If oil pollution did occur, the oil concentrations that would occur in the water column are difficult to predict since the oil volume, mixing duration, mixing intensity, and confinement of the spill are all important but unknown variables that would depend on the specific conditions prevailing at the time of the spill. Further, there are too few quantitative studies on the effects of an oil spill in arctic and subarctic waters that include measurements of oil in the water column for us to state that our laboratory exposure concentrations might be encountered under a field spill situation.

Rice, S. D., J. W. Short, and J. F. Karinen.

1976b. Toxicity of Cook Inlet crude oil and No. 2 fuel oil to several Alaskan marine fishes and invertebrates. In Sources, effects and sinks of hydrocarbons in the aquatic environment, Proceedings of the symposium, American University, Wash., D.C., 9-11 Aug. 1976, p. 395-406. American Institute of Biological Sciences, Washington, D.C.

We used a 96-h static-test method to determine the median tolerance levels (TLm's) of 27 different invertebrate and vertebrate Alaskan marine species exposed to water-soluble fractions (WSF's) of Cook Inlet crude oil and No. 2 fuel oil. Concentrations of oil in the exposure doses of the WSF's were determined by infrared spectrophotometry.

The two different oils were about equally toxic; No. 2 fuel oil was somewhat more toxic than the Cook Inlet crude oil to some of the species. Fish were consistently among the more sensitive species with 96-h TLm's from 0.81 to 2.94 ppm. Some invertebrates were as sensitive as fish, whereas others were quite resistant. Intertidal invertebrates were consistently among the most resistant species.

It appears that Alaskan marine species may be slightly more sensitive than similar species residing in more temperate regions. However, the differences in observed sensitivity may be due to the greater toxicity of oil at lower temperatures (because of greater persistence of hydrocarbons) rather than to actual increases in the sensitivity of the animals.

Rice, S. D., J. W. Short, and J. F. Karinen.

1977a. Comparative oil toxicity and comparative animal sensitivity. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 78-94. Pergamon Press, New York.

The scope of this review is limited to studies dealing with the ability of crude and refined oils to kill marine animals. Emphasis is on the more recent quantitative studies that were not available to earlier reviewers (Evans and Rice 1973; Moore and Dwyer 1974; National Academy of Sciences 1975). This review covers (1) the behavior of oil in water; (2) the methodology problems associated with tests; (3) the comparative toxicity of oil-water mixtures, oils, and components of oils; and (4) the comparative sensitivity of different life stages and species.

Rice, S. D., R. E. Thomas, and J. W. Short.

1977b. Effect of petroleum hydrocarbons on breathing and coughing rates and hydrocarbon uptake-depuration in pink salmon fry. In F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (editors), Physiological responses of marine biota to pollutants, Proceedings, p. 259-277. Academic Press, New York.

1. The water-soluble fraction (WSF) from Cook Inlet and Prudhoe Bay crude oils and No. 2 fuel oil causes similar increases in breathing and coughing rates in pink salmon fry.

2. Breathing and coughing rates increase in proportion to oil concentrations, as measured by ultraviolet but not by infrared spectrophotometry. This suggests that naphthalenes rather than paraffins are responsible for this effect. Significant responses were detected at about 30% of the 96-h TLm.

3. Breathing and coughing rates of pink salmon fry remained above normal during exposure to a constant dose of oil for 72 h.

4. Paraffinic, monoaromatic, and diaromatic hydrocarbons were found in tissues of fish exposed to the WSF of Cook Inlet crude oil. The fish started apparent deputation of the aromatic hydrocarbons during the first 24 h of exposure; this indicates that they can cope with the stress physiologically. Our data support the concept of excretion through the liver-gall bladder-gut.

5. High breathing rates during the first 14 h of exposure, elimination of most aromatic hydrocarbons by 20 h, and the continued high breathing rates during the constant-dose exposure for 72 h indicate that salmon fry can cope with a sublethal exposure to hydrocarbons, but at the cost of an increased metabolic rate. Increased metabolic rates may be detrimental to survival if the stress persists for long periods of time.

Rice, S. D., and S. A. Andrews.

Unpublished manuscript-a. Quantitative comparison between the toxicity of water-soluble fraction of crude oil and a similar mixture of aromatic hydrocarbons to pink salmon and kelp shrimp.

Aromatic hydrocarbons in the water-soluble fraction (WSF) of Cook Inlet crude oil are thought to cause most of the toxicity of the parent oil. To test this hypothesis, we compared the toxicity of the WSF of Cook Inlet crude oil with the toxicity of a mixture of aromatic compounds combined in the same concentrations as those found in the WSF. Pink salmon fry and kelp shrimp were exposed to several concentrations of the oil WSF and the simulated WSF. The oil WSF was three times more toxic to salmon fry and five times more toxic to kelp shrimp than the simulated WSF. Components other than aromatic hydrocarbons in the oil WSF contribute considerably to the toxicity of crude oil.

Rice, S. D., and S. A. Andrews.

Unpublished manuscript-b. Additive and synergistic toxicity of pairs of aromatic hydrocarbons to pink salmon fry.

Aromatic hydrocarbons are generally thought to be the primary contributors to the toxicity of crude oil in tests with marine organisms. Some laboratory evidence, however, indicates that the water-soluble fraction (WSF) of crude oil is more toxic than predicted by the individual toxicities of its component aromatic hydrocarbons. Because synergistic effects between aromatic hydrocarbons are possible, we tested whether there were synergistic effects between benzene and other monoaromatic hydrocarbons in the WSF of Cook Inlet crude oil. Pink salmon fry were exposed for 96 h to several concentrations of each of the following compounds mixed in different ratios with benzene: Toluene; o-, m-, and p-xylene; 1,2,4-trimethylbenzene and 1,3,5-trimethylbenzene; ethylbenzene; and isopropylbenzene. Many of the mixtures were more toxic than an additive model would predict. The greatest interaction was between benzene and p-xylene in the ratio of 1:1.3. This mixture was three times more toxic, as determined from LC50's, than predicted from the additive model. In conclusion, the toxicity of mixtures of monoaromatic hydrocarbons is unpredictable, unless individual combinations are tested, because the total toxicity of mixtures may be greater than the effects of any of them singly.

Short, J. W., and S. D. Rice.

In preparation. Accumulation, deputation, and retention of petroleum-derived aromatic hydrocarbons by four commercially important Alaskan marine animals (pink scallops, pink shrimp, king crab, and pink salmon).

We exposed pink scallops, pink shrimp, king crabs, and pink salmon fry to the water-soluble fraction (WSF) of Cook Inlet crude oil for up to 5 days and determined concentrations of aromatic hydrocarbons in each species. At the end of the exposures, live animals were transferred to clean seawater, and concentrations of aromatic hydrocarbon concentrations in each species were again determined after various intervals of up to 10 days.

During the exposures, naphthalene and methyl-substituted naphthalenes were consistently found in all four species; phenanthrene and methyl-substituted phenanthrenes were found in scallops; and three- and four- carbon aliphatic-substituted benzenes were found in scallops and fry.

Scallops, fry, and shrimp rapidly accumulated aromatic hydrocarbons during exposure to the WSF. After an initial delay of about 10 h, crabs rapidly accumulated aromatic hydrocarbons during exposure to the WSF. All four species had maximum accumulations of aromatic hydrocarbons after 10-48 h of exposure to WSF, and concentrations of aromatic hydrocarbons in the animals were between 33 times (naphthalene in scallops) and 1,024 times (methylnaphthalene in king crabs) the concentration of aromatic hydrocarbon in the WSF.

Each species had a unique pattern for the elimination of aromatic hydrocarbons. The concentrations of aromatic hydrocarbons in scallops declined slowly and incompletely during the 5 days that scallops were in clean seawater. Concentrations of aromatic hydrocarbons in pink shrimp declined rapidly within 24 h after transfer to clean seawater and more slowly thereafter. Concentrations of aromatic hydrocarbons in king crabs rapidly declined to control levels within 4 days after transfer to clean seawater. Concentrations of aromatic hydrocarbons appeared to decline dramatically in pink salmon fry while they were still being exposed to the WSF. The ability of these species to rapidly accumulate aromatic hydrocarbons at low temperatures indicates that their marketability could be impaired if they were exposed to sufficiently oil-polluted water for more than a few hours.

Short, J. W., S. D. Rice, and D. L. Cheatham.

1976. Comparison of two methods of oil and grease determination. In D. W. Hood and D. C. Burrell (editors), Assessment of the arctic marine environment, selected topics, p. 451-462. University of Alaska, Fairbanks, Institute of Marine Science, Occasional Publication No. 4.

A gravimetric method is used by government regulatory agencies for determining levels of oil pollutants in discharge waters. This method involves extraction with an organic solvent, evaporation at elevated temperatures, and gravimetric determination of the residue. The authors compare oil content determined by the gravimetric method with oil content determined by infrared spectrophotometry for toxic water-soluble fractions of Prudhoe Bay and Cook Inlet crude oils and a No. 2 fuel oil.

The gravimetric method is adequate for grease but not for the oils. Recovery of a synthetic grease standard was 98%, whereas the recovery of the three pure oils ranged from 52 to 65% by the gravimetric method. Recovery of all the oils and the grease standard was essentially 100% by the infrared method. The differences between the two methods are ascribed to significant losses of volatile compounds from the oils during the evaporation step of the gravimetric method.

Gravimetric estimates of oil in toxic concentrations of water-soluble fractions (WSF's) ranged from 0 to 36% of those determined by the infrared method. Oil content of the No. 2 fuel oil WSF's was below detectable limits of the gravimetric method (1.5 mg/liter). Four-day median tolerance limits of shrimp (Eualus fabricii) and scallops (Chlamys rubida), as evaluated by the infrared WSF's for the three oils, were between 0.25 mg/liter (No. 2 fuel oil) and 3.82 mg/liter (crude oils).

It is concluded that the gravimetric method is sensitive to heavier compounds, but these have only a casual relationship to acute toxicity. Concentrations of oil in water known to have adverse effects are much lower than can be detected by the standard gravimetric method. When oil concentrations in water are to be measured and correlated with chemical toxicity, the gravimetric procedure should be supplemented with a method specific for the more soluble and volatile components.

Short, J. W., J. A. Gharrett, S. Kern, and S. D. Rice.

In preparation. Rapid uptake and deputation of aromatic hydrocarbons in coonstripe shrimp larvae.

Coonstripe shrimp larvae were exposed to ^{14}C-labeled toluene, ^{14}C-labeled naphthalene, and ^{3}H-labeled 2-methylnaphthalene for 3 h. Larvae were kept in glass tubes with screen bottoms. The tubes were then placed in static seawater solutions of aromatic hydrocarbons (either toluene alone or the two naphthalenes together). At regular intervals, groups of larvae were removed, rinsed, and burned in an oxidizer. The residues were collected, and radioactivity was measured by liquid scintillation to determine the amount of aromatic hydrocarbons taken up by the larvae. All unsampled larvae were moved to uncontaminated seawater after 3 h, and sampling continued to trace the deputation of aromatic hydrocarbons.

In <10 rein, the larvae accumulated many times the water concentrations of the aromatic hydrocarbons. About 1 h after the start of exposures, concentrations of aromatic hydrocarbons in the animals stabilized. The concentration of toluene (1.4 ppm) in the animals was 10 times the exposure concentration; the concentration of naphthalene (0.075 ppm) was nearly 100 times the exposure concentration; and the concentration of 2-methylnaphthalene was nearly 500-1,000 times the exposure concentration.

The larvae depurated hydrocarbons rapidly for the first 9 h after being moved to clean seawater but only slowly thereafter. Twenty-four hours after deputation began, all larvae still contained concentrations of hydrocarbons that were higher than the original exposure concentrations. After 48 h in clean seawater, they depurated about 50% of the hydrocarbons remaining after 24 h of deputation in clean seawater.

In similar but less extensive tests, Stage I and Stage II shrimp larvae took up similar amounts of hydrocarbons, and dead larvae took up nearly as much hydrocarbon as live ones. The uptake mechanisms for hydrocarbons in shrimp must, therefore, be passive.

Stickle, W. B., Jr., S. D. Rice, and D. A. Moles.

1984. Bioenergetics and survival of the marine snail, Thais lima during long-term oil exposure. Mar. Biol. (Berl.) 80:281-289.

The carnivorous snail Thais lima was fed Mytilus edulis during a 28-d exposure to the water soluble fraction (WSF) of Cook Inlet crude oil. The LC50 of T. lima declined from >3,000 ppb aromatic hydrocarbons on day 7 to 818 ± 118 ppb aromatic hydrocarbons on day 28. The LC50 of M. edulis declined from >3,000 ppb aromatic hydrocarbons on day 7 to 1,686 ± 42 ppb on day 28. Predation rate declined linearly with increasing aromatic hydrocarbon concentration up to 302 ppb; little predation occurred at 538 ppb and none at 1,160 or 1,761 ppb. Snail absorption efficiency averaged 93.5% and did not vary as a function of WSF dose. Total energy expenditure (R + U) increased at 44 ppb aromatics and declined at lethal WSF exposures. At sublethal WSF exposures, percentages of total energy expenditure were: respiration (87%), ammonia excretion (9%) and primary amine loss (4%). These percentages did not vary as a function of WSF dose or time. Oxygen:nitrogen ratios were not affected by WSF concentration or time and indicated that T. lima derived most of its energy from protein catabolism. The uptake of aromatic hydrocarbons into the soft tissues of snails and mussels was directly related to the WSF concentration. Naphthalenes accounted for 67 to 78% of the aromatic hydrocarbons in T. lima and 56 to 71% in M. edulis. The scope for growth was negative above 150 ppb WSF aromatic hydrocarbons and above 1,204 ppb soft-body aromatic hydrocarbons. These snails were physiologically stressed at an aromatic hydrocarbon concentration that was 19% of the 28-d WSF LC50 (818 ± 118 ppb) and/or 48% of the 28-d LC50 of soft tissue aromatics (2,502 ppb).

Stickle, W. B., Jr., S. D. Rice, C. Villars, and W. Metcalf.

In press. Bioenergetics and survival of the marine mussel, Mytilus edulis, during long-term exposure to the water-soluble fraction of Cook Inlet crude oil. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Symposium on pollution and physiology of marine organisms, Mystic, Corm., Nov. 1983. University of South Carolina Press.

The survival, energy budget (scope-for-growth), and oxygen:nitrogen ratios were determined in the filter-feeding mussel Mytilus edulis during exposures (flow through) to the seawater-soluble fraction (WSF) of Cook Inlet crude oil (30°/$_{oo}$ salinity and 10"C). Mussels were exposed for up to 28 days to concentrations of WSF as high as 2.1 ppm aromatic hydrocarbons. Scope-for-growth was calculated from feeding rate of M. edulis on Phaeodactylum tricornutum at 1.2 x 10^4 cells ml^{-1}, absorption efficiency, oxygen consumption, and ammonia excretion rates of six groups of large (2-5 cm) mussels that were exposed to O, 0.09, 0.21, 0.38, 0.60, and 1.1 ppm aromatic hydrocarbons. Survival and tissue concentrations of aromatic hydrocarbons were determined on days O, 7, 14, 21, and 28 of the experimental period.

The LC50 of mussels declined during the 28-day experiment from >2.5 ppm of aromatic hydrocarbons on day 7 to 2.5 ppm ± 0.71 (mean and 95% C.I.) on day 14, 1.9 ppm ± 0.48 on day 21, and 1.4 ppm ± 0.30 on day 28. Feeding rate was unaffected at O, 0.09, and 0.21 ppm aromatic hydrocarbons and varied between 408 ml ± 105 ml (SE) and 939 ml ± 126 ml·(g dry weight of algae eaten)$^{-1}$·h^{-1}. The feeding rate of mussels exposed to 0.38 ppm aromatic hydrocarbons declined significantly after 14 days of exposure, whereas the feeding rates of mussels exposed at 0.60 and 1.1 ppm aromatic hydrocarbons were significantly lower than the control rate at all sampling times. Absorption efficiency varied between 51% and 84% and was unaffected by exposure time at concentrations ≤0.38 ppm aromatic hydrocarbons. Absorption efficiency declined to 43% on day 28 in mussels exposed to 0.60 ppm aromatic hydrocarbons and to 41% and 48% on days 21 and 28 in mussels exposed at 1.1 ppm. Caloric consumption due to respiration and ammonia excretion varied between 0.52 calories·mussel^{-1}·h^{-1} and 1.10 calories-mussel^{-1}·h^{-1} and did not fluctuate as a function of exposure time at any dose. Respiration accounted for more than 87% of caloric expenditure at all times. Oxygen:nitrogen ratios did not vary as a function of

exposure time or exposure concentration and ranged between 13.6 ± 1.3 (mean and 95% C.I.) and 50.0 ± 26.1 and indicated increased reliance on protein catabolism. Mussels had spawned just before the start of our experiment, however, and were presumably reabsorbing gametes during the experiment. Bioaccumulation of aromatic hydrocarbons was dose related.

Scope-for-growth was unaffected by exposure time at 0, 0.09, and 0.21 ppm aromatic hydrocarbons and varied between 1.21 and 3.87 $cal \cdot mussel^{-1} \cdot l$. Scope-for-growth of mussels exposed to 0.38 ppm aromatic hydrocarbons declined significantly from earlier values on days 21 and 28, whereas scope-for-growth of mussels exposed to 0.60 and 1.1 ppm was significantly lower than the day-0 value on all sampling dates during exposure. The lowest aromatic hydrocarbon concentration that reduced scope-for-growth was 0.38 ppm on day 21 of exposure; this concentration represents 19.7% of the 21-day LC50.

Stickle, W. B., T. D. Sabourin, and S. D. Rice.

1982. Sensitivity and osmoregulation of coho salmon, Oncorhynchus kisutch exposed to toluene and naphthalene at different salinities. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Physiological mechanisms of marine pollutant toxicity, p. 331-348. Academic Press, New York.

Coho salmon (Oncorhynchus kisutch) smelts were more sensitive to toluene and naphthalene in seawater than in fresh water. Tolerance dropped linearly from 0 through 10, 20, and 30°/₀₀ S. Smelt tolerances at 30°/₀₀ were 54% and 63% of the 48-h TLm in fresh water for toluene and naphthalene, respectively. Smelt tolerances to toluene and naphthalene were the same after 12, 22, and 42 days of acclimation to seawater as they were after only 1 day of acclimation. The increase in sensitivity was not transient nor did it appear related to acclimation-stress because the smelts gained 30% in weight in 42 days.

Toluene and naphthalene affected serum osmolality and ions but only at the lethal concentrations of 100 and 130% of the 48-h TLm. At those exposure concentrations, osmolality, Na^+, and Cl drifted with the diffusion gradient, decreased in freshwater smelts, and increased in seawater smelts. At the same concentration, K^+ concentrations in the serum increased, even in freshwater smol ts, indicating cellular damage. Exposures of 70% of the 48-h TLm had no effect on serum osmolality or ions. Consequently, we conclude that the increase in sensitivity of smelts in seawater is not related to a failure in ion-regulating ability, but rather the loss of ion-regulating ability at lethal exposures is symptomatic of toxic actions elsewhere. The cause of increased sensitivity of smelts in seawater is not transient and remains unknown.

Taylor, T. L., and J. F. Karinen.

1977. Response of the clam, Macoma balthica (Linnaeus), exposed to Prudhoe Bay crude oil as unmixed oil, water-soluble fraction, and oil-contaminated sediment in the laboratory. In D. A. Wolfe (editor), Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems, Proceedings of a symposium, 10-12 Nov. 1976, Seattle, Wash., p. 229-237. Pergamon Press, New York.

The small clam, Macoma balthica (Linnaeus 1758), will likely be subjected to oil slicks layered on the mud and to water-soluble fractions (WSF's) of crude oil or oil-contaminated sediment. Groups of adult clams in or on their natural sediment were exposed in flow-through aquaria at 7-12°C to various concentrations of Prudhoe Bay crude oil layered on the mud surface, the WSF of the crude oil, and oil-treated sediment (OTS).

Gentle settling of crude oil over clam beds had negligible effects on clams observed for 2 months. The WSF and OTS of Prudhoe Bay crude oil inhibited burrowing and caused clams to move to the sediment surface. Responses were directly proportional to concentrations of WSF or amount of OTS. The l-h and 72-h effective median concentrations of the WSF for the responses of burrowing by unburied clams and surfacing by buried clams were 0.234 and 0.367 ppm naphthalene equivalents, respectively. The interpolated amount of OTS needed for a 50% surfacing response within 24 h was 0.67 g OTS cm^{-2}.

Although short-term exposures of clams to the WSF of crude oil and OTS caused few deaths, behavioral responses of clams to oil may be of great importance to their survival in the natural environment. In these laboratory tests, many of the clams recovered, but in nature, clams that come to the sediment surface may be eaten by predators or die from exposure.

Thomas, R. E., and S. D. Rice.

1975. Increased opercular rates of pink salmon (Oncorhynchus gorbuscha) fry after exposure to the water-soluble fraction of Prudhoe Bay crude oil. J. Fish. Res. Board Can. 32:2221-2224.

The opercular rates of pink salmon (Oncorhynchus gorbuscha) fry were measured during 24-h exposure to sublethal concentrations of the water-soluble fraction (WSF) of Prudhoe Bay crude oil. Opercular rates increased significantly for as long as 9 and 12 h after exposure to WSF's prepared from oil-water solutions of 2.83 and 3.46 ppm. The increases in rates were proportional to increases in dose. Recording changes in opercular rates appears to be a suitable method for detecting sublethal physiological effects of hydrocarbon stress because the observed changes occurred at approximately 20% of the 96-h LC_{50}.

Thomas, R. E., and S. D. Rice.

1979. The effect of exposure temperatures on oxygen consumption and opercular breathing rates of pink salmon fry exposed to toluene, naphthalene, and water-soluble fractions of Cook Inlet crude oil and No. 2 fuel oil. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and F. J. Vernberg (editors), Marine pollution: functional responses, p. 39-52. Academic Press, New York.

Oxygen consumption and breathing rates of fry exposed to toluene and naphthalene began to increase immediately upon exposure and declined in later hours during exposure. Breathing rate reached maximum response values at 2 or 4 h, whereas oxygen consumption rates were greatest at 6 or 8 h of exposure.

All three concentrations of naphthalene (107%, 70%, and 45% of the 24-h median tolerance limit) resulted in significant increases in the opercular breathing rate of pink salmon fry ($P < 0.01$); whereas, of the four toluene concentrations (94%, 69%, 45%, and 30% of the 24-h TLm), only the two highest resulted in a significant increase in the breathing rate. Breathing rate response was linear with dose.

Although relatively few fish were used in the studies of oxygen consumption, the pattern of increased oxygen consumption along with increased breathing rates in each of the exposures indicates that increased breathing rate of pink salmon fry reflects increased energy demands. Oxygen consumption was greatest at about 6 h of exposure, about 4 h after the occurrence of maximum breathing rates. Apparently, as the fry became physiologically acclimated to the stress of the toxicant, they increased the efficiency of oxygen extraction, thus decreasing the need to move water across the gills and subsequent expenditure of energy.

The increase in breathing rates of naphthalene-exposed fry over control fry were much greater at the low temperature of 4° than 12°C. Control fry at 4°C had lower metabolic rates. However, the breathing rate response to hydrocarbons, as a percent of controls, was much larger at 4° than at 12°C, indicating that hydrocarbon exposures at 4*C are more stressful than equivalent exposures at 12°C.

Thomas, R. E., and S. D. Rice.

1981. Excretion of aromatic hydrocarbons and their metabolizes by freshwater and seawater Dolly Warden char. In J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg (editors), Biological monitoring of marine pollutants, p. 425-448. Academic Press, New York.

The gills were the most important pathway for excretion of carbon-14 from ^{14}C-labeled naphthalene. Most of the carbon-14 excreted by the gills was still attached to the parent compound. About 10% of the excreted carbon-14 appeared in the cloacal chamber, mostly as metabolizes. Less than 1% of the total carbon-14 was excreted in the urine, predominantly as metabolizes.

Tissues retained a significant amount of carbon-14 at 24 h. Although muscle contained large amounts of carbon-14 because of its mass, the gall bladder had the highest specific activity. The brain also retained significant quantities of carbon-14.

Although more ^{14}C-labeled toluene was excreted and metabolized than ^{14}C-labeled naphthalene, more ^{14}C-labeled naphthalene was retained in the tissues. A lower percentage of the carbon-14 was recovered in ^{14}C-labeled naphthalene metabolizes than in ^{14}C-labeled toluene metabolizes.

Seawater and freshwater Dolly Varden char excreted similar amounts of carbon-14; however, the percentage of metabolizes in the excretions and tissues of seawater fish was lower than the percentage of metabolizes in excretions and tissues of freshwater fish. For example, we recovered greater amounts of carbon-14 with a lower percentage of metabolizes from the brain-spinal cord of seawater fish than from the brain-spinal cord of freshwater fish--possibly explaining why seawater Dolly Warden char are more sensitive to aromatic hydrocarbons and the water-soluble fraction of oil than freshwater Dolly Varden.

Thomas, R. E., and S. D. Rice.

1982. Metabolism and clearance of phenolic and mono-, di-, and polynuclear aromatic hydrocarbons by Dolly Varden char. In W. B. Vernberg, A. Calabrese, F. P. Thurberg, and J. F. Vernberg (editors), Physiological mechanisms of marine pollutant toxicity, p. 161-176. Academic Press, New York.

Although the pathways of elimination of hydrocarbons have generally been identified, the relative importance of each route for compounds of different molecular weight and polarity is not known. In this study, we compared and determined the effects of molecular weight and polarity on the clearance of several phenolic and aromatic hydrocarbons from gills and other excretory pathways of Dolly Varden char (Salvelinus malma) in seawater. We also examined the distribution of parent hydrocarbons and metabolizes in tissues after 24 h of exposure. Dolly Varden char were force-fed gelatin capsules containing a ^{14}C-labeled hydrocarbon and then placed in a split-chamber box for 24 h to separate gill excretions from cloacal excretions.

Size of the hydrocarbon appeared to be the most critical factor in excretion of hydrocarbons by the gills. The gills of fish can easily excrete phenolic and mononuclear aromatic compounds. Some naphthalene was excreted from the gills, but virtually none of the polynuclear aromatic hydrocarbons were excreted from the gills. Even though the partition coefficients (log of octanol/water partition) of phenol and cresol are about 1/10 of toluene, these similar-sized hydrocarbons were excreted from the gills in approximately equal amounts.

Polar phenolic compounds were excreted into the cloacal chamber but not toluene or the large polynuclear aromatic hydrocarbons. Partition coefficient is apparently a more important factor than size in excretion of hydrocarbons into the cloacal chamber because the excretion of phenol and cresol into the cloacal chamber was more than 10 times that of similar-sized toluene.

The excretion of the largest hydrocarbons that were tested, anthracene and benzo[a]pyrene, was minimal in 24 h (3.2% and 1.2%, respectively). These compounds were slowly absorbed from the gut probably because they are relatively nonpolar and have a high partition coefficient (octanol/water), therefore, are more difficult to remove from a lipid matrix (membrane). The mobility of these compounds between tissues is limited, and they probably have

to be metabolized before excretion. Consequently, metabolism in the liver and secretion into the bile is probably the most important pathway for excretion of large molecular weight hydrocarbons; however, this is a relatively slow process that takes much longer than 24 h.

Thomas, R. E., and S. D. Rice.

In press. Effect of previous exposure to naphthalene in the water column on the subsequent metabolism of dietary naphthalene by Dolly Varden char, Salvelinus malma. Symposium on pollution and physiology of marine organisms, Mystic, Corm., Nov. 1983.

Dolly Varden (Salvelinus malma) acclimated to seawater (30°/$_{oo}$) at 4°C were exposed to naphthalene in the water of their holding tank. The fish were fed ^{14}C-labeled naphthalene in a gelatin capsule that was placed in their stomach. Concentrations of parent hydrocarbon and metabolizes in the gall bladder (bile), liver, central nervous system, and muscle were determined 24 h after administration of the capsule.

The conversion of dietary naphthalene to tissue metabolizes was dependent on the concentration of naphthalene in the exposure water. Generally, the percent of carbon-14 in the metabolize fraction of the tissues increased after 48-h exposures as the concentration of the naphthalene in the water exposure increased from 25% of the 24-h LC50 to 75% of the 24-h LC50.

Groups of fish were also exposed for 48 h to naphthalene at 75% of the 24-h LC50, and then transferred to clean water for 12 or 24 h periods before ^{14}C-labeled naphthalene was placed in their stomachs. Decreased percentages of carbon-14 were recovered in the metabolize fraction from the tissues as the time in clean water increased. After 24 h in clean water, the percent radioactivity in the metabolize fraction of animals previously exposed to naphthalene was similar to that of animals that were not exposed to naphthalene.

Length of the previous exposure to naphthalene also influenced the amount of carbon-14 recovered in the metabolize fraction. Although a previous exposure of 48 h resulted in a significant increase in tissue metabolizes, an exposure of only 24 h did not. The significance of enzyme induction resulting from a previous exposure to petroleum hydrocarbons is discussed.

Thomas, R. E. and S. D. Rice.

In preparation-a. Effect of environmental temperature on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden, Salvelinus malma.

Dolly Varden (Salvelinus malma) acclimated to seawater (300/0.) or fresh water at 4° or 12°C were force-fed gelatin capsules containing ^{14}C-labeled toluene or ^{14}C-labeled naphthalene. Concentrations of parent hydrocarbons and metabolizes in the gall bladder (bile), liver, central nervous system, and muscle were determined 12, 24, and 48 h after initiation of exposure.

In fresh water, the temperature of 12°C promoted the absorption of both ^{14}C-labeled hydrocarbons from the stomach into tissues, and fish had more of the administered radioactivity recovered in the tissues than fish exposed at 4"C. Liver was the only tissue that did not follow this trend. More carbon-14 was recovered from the liver of fish exposed to naphthalene or toluene at 4° than at 12°C but only at the 12-h exposure. A similar pattern was observed for fish acclimated to seawater. More carbon-14 was recovered from the tissues at 12°C than at 4°C. However, more carbon-14 was recovered from fish acclimated to seawater than from fish acclimated to fresh water, regardless of temperature.

Significantly ($P < 0.01$) more carbon-14 was recovered in the metabolize fraction after 12°C exposures to naphthalene in fresh water than after 4°C exposures. This was true for all tissues at 12, 24, and 48 h after exposure in fresh water. More carbon-14 was found in the metabolize fraction after 12°C exposures to toluene in fresh water than after 4°C exposures although the differences were not as pronounced as in naphthalene exposures. Temperature had a similar effect on the percent carbon-14 recovered in the metabolize fraction after fish were acclimated to seawater; however, the effect was not as pronounced as in fish exposed in fresh water. Liver, nerve, and muscle tissues removed from the fish exposed to toluene in seawater at 12 and 24 h contained significantly ($P < 0.01$) more carbon-14 in the metabolize fraction at 12°C than at 4"C. For animals exposed to naphthalene, however, concentrations of tissue metabolizes were similar for 4° and 12°C exposures until after 48 h of exposure.

Generally, compared to fish acclimated to 12°C, fish acclimated to 4°C had lower concentrations of parent hydrocarbon and its metabolizes in the

bile, liver, central nervous system, and muscle, regardless of salinity or toxicant. The effect of temperature, however, was less pronounced in fish acclimated to seawater. The differences in the effect of temperature are undoubtedly caused by many temperature-sensitive processes, such as absorption, metabolism, retention, and excretion. The role of temperature in altering the rates of these processes is discussed.

Thomas, R. E., and S. D. Rice.

In preparation-b. Effect of salinity on tissue incorporation and metabolism of toluene and naphthalene by Dolly Varden char, Salvelinus malma.

Dolly Varden char maintained at 12°C in seawater (30°/00) or fresh water were force-fed gelatin capsules containing ^{14}C-labeled toluene or ^{14}C-labeled naphthalene. Tissue concentrations of parent hydrocarbon and metabolizes were determined at 12, 24, or 48 h after initiation of the exposure. Tissues sampled included gall bladder (bile), liver, central nervous system, and muscle.

Consistently higher concentrations of toluene or naphthalene were recovered in the tissues of animals exposed in seawater than in the tissues of animals exposed in fresh water. Generally, the amount of labeled hydrocarbon recovered from the tissues increased 48 h after exposure; however, the magnitude of the increase was dependent on the tissue sampled, the compound, and whether the fish were exposed in seawater or fresh water.

Significantly ($P < 0.01$) more carbon-14 was recovered as polar metabolizes from the animals exposed to naphthalene in fresh water than from animals exposed to naphthalene in seawater. More metabolizes of toluene were also found in tissues of fish exposed in fresh water than in fish exposed in seawater; however, the differences were not nearly as great as in the naphthalene exposures. Generally, the percentage of carbon-14 recovered as metabolizes increased with time for both compounds in both the freshwater and seawater exposures. Again, the magnitude of increase was dependent on type of tissue, the hydrocarbon administered, and freshwater or seawater exposure. The salinity of exposure does affect the accumulation and metabolism of aromatic hydrocarbons in fish and may explain why salmonids in seawater are more sensitive to aromatic hydrocarbons than salmonids in fresh water.

APPENDIX B.

SCIENTIFIC AND COMMON NAMES OF 64 ALASKAN SPECIES THAT HAVE BEEN EXPOSED TO HYDROCARBONS BY RESEARCHERS AT THE AUKE BAY LABORATORY

Annelids

Harmothoe imbricata	scale worm
Nereis vexillosa	mussel worm

Nemerteans

Lineus vegetus	brown ribbon worm
Paranemertes peregrina	purple ribbon worm

Crustaceans

Acanthomysis pseudomacropsis	mysid
Balanus glandula	barnacle
Idothea wosnesenskii	isopod
Anonyx nugax	amphipod
Boeckosimus nanseni	amphipod
Gammaracanthus loricatus	amphipod
Orchomene pinquis	amphipod
Pandalus borealis	pink shrimp
Pandalus danae	dock shrimp
Pandalus goniurus	bumpy shrimp
Pandalus hypsinotus	coonstripe shrimp
Eualus fabricii	scooter shrimp
Eualus suckleyi	kelp shrimp
Crangon alaskensis	grass shrimp
Pagurus hirsutiusculus	hairy hermit crab
Paralithodes camtschatica	red king crab
Cancer magister	Dungeness crab
Chionoecetes bairdi	Tanner crab
Hemigrapsus nudus	purple shore crab

Molluscs

Colus halli	Hall's colus
Colus jordani	
Littorina sitkana	Sitka periwinkle
Margaritas pupillus	purple margarite
Neptunea lyrata	ridged whelk
Thais lima	file periwinkle
Collisella (Notoacmaea) pelta	shield limpet
Collisella (Notoacmaea) scutum	plate limpet
Ischnochiton stelleri	giant chiton, gumboot
Katharina tunicata	leather chiton
Mopalia ciliata	ciliated chiton
Tonicella lineata	lined chiton
Chlamys hericius	pink scallop
Macoma balthica	Baltic clam
Protothaca staminea	littleneck clam
Mytilus edulis	blue mussel

Echinoderms

Leptasterias hexactis	six-armed starfish
Evasterias troschelii	seastar
Eupentacta quinquesemita	white cucumber
Cucumaria vegae	tarspot cucumber
Strongylocentrotus droebachiensis	green sea urchin

Fishes

Anoplarchus purpurescens	cockscomb prickleback
Aulorhynchus flavidus	tube snout
Boreogadus saida	Arctic cod
Clupea harengus pallasi	Pacific herring
Cottus cognatus	slimy sculpin
Eleginus gracilis	saffron cod
Gasterosteus aculeatus	threespine sticklebacks
Limanda aspera	yellowfin sole
Myoxocephalus polyacanthocephalus	great sculpin
Oncorhynchus gorbuscha	pink salmon
Oncorhynchus kisutch	coho salmon
Oncorhynchus nerka	sockeye salmon
Oncorhynchus tshawytscha	chinook salmon
Oncottus hexacornis	sculpin
Salvelinus alpinus	Arctic char
Salvelinus malma	Dolly Varden
Theragra chalcogramma	walleye pollock
Thymallus arcticus	Arctic grayling
Pholis laeta	crescent gunnel
Platichthys stellatus	starry flounder